Title: Individual heterozygosity predicts translocation success in threatened desert tortoises

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Abstract: Anthropogenic environmental modification is placing as many as one million species at risk of extinction. One management action for reducing extinction risk is translocation of individuals to locations from which they have disappeared or to new locations where biologists hypothesize they have a good chance of surviving. To maximize this survival probability, the standard practice is to move animals from populations that are as nearby as possible which contain presumably related individuals. Here we report the first empirical test of this conventional wisdom by analyzing a genomic data set for 166 translocated desert tortoises (*Gopherus agassizii*) that either survived, or died, over two decades. We used genomic data to infer the geographic origin of translocated tortoises and found that individual heterozygosity predicted tortoise survival, but translocation distance or geographic unit of origin did not. Our results suggest a relatively simple indicator of the likelihood of a translocated individual's survival – heterozygosity.

One Sentence Summary:

Landscape genomics and long-term field data show that individual heterozygosity predicts post-translocation survivorship of threatened desert tortoises.

Main Text: In a world of rapid environmental change, habitat loss, and species endangerment, translocation of individual plants and animals is becoming increasingly common as a conservation strategy of last resort. For both animals and plants, the long-term success of

translocations is often dismal (*1-3*). There are many possible explanations for this low success, and conservation biologists have proposed several guidelines for improving translocation outcomes, including limiting translocation distances and only exchanging individuals from within the same genetic units to minimize outbreeding depression (*4*). Though levels of inbreeding and heterozygosity have long been linked to individual survival and fitness (*5-7*), the relationship between these and translocation success has received little attention. Here we exploit a long-term data set for threatened Mojave desert tortoises to generate genomic profiles for 166 tortoises and link those data to individual post-translocation survival.

Mojave desert tortoises (*Gopherus agassizii*) are widely distributed members of the Mojave and Sonoran Desert ecological communities west and north of the Colorado River in California, Nevada, Utah, and Arizona, USA (Fig. 1). Although the tortoise is a ubiquitous member of the relatively intact desert ecosystem, decreasing population trends led to the early listing of the species as threatened under the U.S. Endangered Species Act (*8*). Part of the species' recovery plan includes translocating tortoises salvaged from harmful anthropogenic activity and habitat destruction to new sites to augment declining populations (*9,10*).

Since the establishment of a recovery plan (*9*), genetic (*11,12*) and genomic (*4,13*) studies have quantified native population structure within Mojave Desert tortoises and have consistently shown that the greatest axis of variation separates the Upper Virgin River and Northeastern Mojave Recovery units (hereafter "northern Mojave") from the rest of the species' distribution (hereafter "southern Mojave", Fig. 2). Additional fine-scale population structure has been documented within these two regions, leading some researchers to recommend translocations only within these genetically defined populations (*4,11,14,15*). Others have recommended limiting translocations to specified distances (200–276 km) based on spatially distributed genetic

structure (*16*). As is generally the case, these recommendations have assumed that moving animals within, but not between, genetic units (major clades or genetically related metapopulations) should be the guiding principle.

Following the accumulation of hundreds of displaced tortoises at the Desert Tortoise Conservation Center (DTCC) in 1996, the 100-km2 Large-Scale Translocation Site (LSTS) was established. The LSTS is located in the Ivanpah Valley near Jean, NV within the natural range of the tortoise, and is surrounded by either a tortoise-barrier fence or relatively inhospitable mountains (Fig. 1; Fig. S1). Because the majority of the tortoises Received at the DTCC were captives, many from Nevada's free pet tortoise pickup program, most individuals lacked reliable information on their native site of origin. Between 1997 and 2014 approximately 9105 tortoises (~50.2% of which were adults) of unknown provenance were translocated to the LSTS, where they intermingled with an estimated 1450 adult local tortoises that were natural residents at the site (*17*). Most native and translocated tortoises in the LSTS have since died, consistent with steep declines in neighboring populations, and likely furthered by high post-translocation densities and less comprehensive health screening during the first decade of the translocation program. However, roughly 350 adults were estimated via line-distance surveys to be alive in 2015 (*18*).

By 2016, there were three classes of LSTS tortoises — known-living and known-dead translocated individuals, and unmarked individuals presumed to be pre-translocation residents. For simplicity we refer to these as living, dead, and resident, respectively. Because no information is available on the origins of translocated tortoises, we generated RADseq (Restriction site Associated DNA sequencing) genomic data and used these data to infer the geographic origins of a set of living and dead tortoises by comparing them to 270 low-coverage

Mojave desert tortoise genomes that were field-collected from across the species' range (*13*; Fig. 1).

By comparing living and dead tortoises from the LSTS, we explicitly address three questions central to assisted migration and genetic rescue efforts. First, do tortoises from more distant localities have lower survival fitness than those from nearby sites of origin? Second, do within-genetic unit (northern or southern Mojave) translocations enjoy greater survival than those that cross this primary genetic boundary? Third, are tortoises with higher overall heterozygosity, measured at deeply sequenced RAD loci, more likely to survive than less genetically variable individuals?

Mapped RADseq reads from the LSTS tortoises contained 6,711,580 of the 36,138,619 SNPs found among 270 low-coverage Mojave desert tortoise genomes (*13*). We empirically evaluated several approaches to infer the place of origin of LSTS tortoises (*18*). Placing 12 known-origin calibration samples at the location of their genetic nearest-neighbor resulted in a mean error of 61.7 km (SD = 60.2) from their true origin. A multi-individual, centroid-based placement approach using the eight closest genetic relatives reduced the mean placement error to 41.7 km (SD =25.0). Finally, the optimal combined approach resulted when individuals with heterozygosity (π) values < 0.0020 were placed with their closest genomic match (presumably their closest relative), and individuals with π values > 0.0020 were placed at the centroid of their closest eight genetic relatives. This combined approach resulted in a mean error of 35.6 km (SD = 27.7). The combined method is thus more accurate, but that accuracy may result from overfitting a complex model with only 12 calibration animals. Countering this concern, we note that: 1) 87% of all LSTS tortoises had π values < 0.0020, and so were geolocated based only on their genetic nearest neighbors; and, 2) given the very low coverage (~1.5x) of our 270 reference

tortoise data set, we expected that more heterozygous LSTS tortoises would be difficult to associate with a true closest relative because heterozygosity is underestimated at ~1.5x coverage. Hence, averaging across a set of close matches should outperform a single match for those relatively heterozygous individuals. Because the dual method had the lowest combined error for the 12 calibration samples, we used it to determine the geographic and genetic provenance of all LSTS tortoises.

We calculated probable geographic origins for 166 living and dead tortoises that were matched for release year and sex (Fig. 2). Sixty-eight of the 79 living, and 78 of the 87 dead LSTS tortoises were genomically placed in the geographically proximal northern Mojave genetic unit, and the remainder in the more distant southern Mojave unit (*13*). We found no difference in the proportion of northern vs southern Mojave desert tortoises that survived/died after translocation (χ^2 (df =1, N=166) =1.18, P = 0.28). We also detected no effect of the geographic distance of site-of-origin from the LSTS for individuals that died or survived following translocation (Fig. 3b; P = 0.83).

In contrast, we found that LSTS translocated survivors had much higher individual heterozygosity when they were compared to those that died (Fig. 3a; mean π of living tortoises = 0.00180, mean π of dead tortoises = 0.00146; P = 0.00000005), indicating that individual genetic diversity predicted translocation success after accounting for release year and sex. The mean heterozygosity of the survivors was 23.09% percent greater than that of a matched set of tortoises that died over the same period. Although the importance of genetic diversity, or its presumed proxy, population size, of stock populations for translocation has been the subject of a few recent studies, this small body of work has yielded contradictory results on the role of population-level variation in translocation success, with some evidence for negligible importance (*19-21*) and

other evidence for substantial importance (*22*,*23*). This lack of the consistency may stem from the assumption that population-level diversity is an accurate proxy for individual heterozygosity, which has not been tested. Our results demonstrate that individual heterozygosity, rather than population size or overall diversity, is a key, easily measured metric for predicting translocation success.

To explore the possibility that our observed relationship between post-translocation survival and heterozygosity is an artifact of sample age or condition, we confirmed that read depth, sample age, influence of outlier loci, and library complexity are not correlated with heterozygosity (18), and conclude that heterozygosity itself is a strong indicator of post-translocation survival. We are not suggesting that individual heterozygosity should be the only criterion for deciding on the individuals to translocate—local ecology, disease exposure history, and individual condition are some of the other factors that are often critical, and we stress the importance of verifying these results in other systems. We also emphasize that our RAD data, while extensive, is only a proxy for the entire genome, and that additional studies with high-coverage whole genome resequencing could help determine whether survivorship is linked to runs of homo/heterozygosity, level of individual inbreeding, or specific loci under strong selection. Regardless, one of the advantages of individual heterozygosity is that it can now be easily and economically measured, with reduced representation approaches or at the whole genome level for most organisms, making it a particularly attractive tool for managers and decision-makers.

Our analysis of LSTS survivors and mortalities, combined with detailed, landscape genomic data for the entire species, indicates that matching the geographical provenance of translocated tortoises to their new site had virtually no predictive power in determining survival

fitness over a decadal timescale. However, individual heterozygosity did, with more heterozygous individuals out-surviving less heterozygous ones. While the overall benefits of genome-level variability have long been suggested as a key fitness component (*7*,*24*), we were able to use long-term field data to directly show this benefit under natural conditions. Although field observations have confirmed living status for only 3.8% of translocated tortoises, our data indicate that even under these severe conditions, the more variable tortoises out-survived their less variable conspecifics, suggesting that these genetically variable individuals were better able to survive following translocation. To confirm that individual heterozygosity is responsible for the increase in survival requires understanding the proximate reasons for the massive mortality seen in both translocated and resident tortoises, and we currently lack that knowledge. Long-term monitoring of two tortoise populations adjacent to the LSTS between 2004 and 2014 found annual mean declines of 7.4% and 9.2% occurred between 2004 and 2014 (*25*), corresponding to a roughly 57% – 65% population reduction over 11 years. These declines, although not fully understood, are at least partially attributed to severe regional drought (*26*) which has been associated with sharp increases in mortality of Mojave desert tortoises (*27-29*). Given our current understanding, we can only speculate that drought, combined with high post-release densities, disease, and/or the ecological disruption associated with translocation may be contributing to the high LSTS mortalities.

However, even without a proximate mechanism, our results suggest that an optimal strategy of assisted migration could be to prioritize moving the most genomically variable individuals rather than current practice based solely on geographic or genetic similarity. Given the future climate and anthropogenic changes anticipated across the region, assisted migration will likely be a key component of management of desert tortoises and many other declining or

endangered species, and our data indicate that targeting the most variable individuals can enhance the success of this work. Future research aimed at understanding the proximate reasons for this increased survival at the genetic and physiological level constitutes an important next step for more efficient conservation-based translocation success.

References and Notes:

1. B. B. Beck, L. G. Rappaport, M. S. Price, A. Wilson, "Reintroduction of captive-born animals" in *Creative Conservation: Interactive Management of Wild and Captive Animals*, P. J. S. Olney, G. M. Mace, A. T. C. Feistner, Eds. (Chapman and Hall, London, 1994) pp. 265–284.

2. J. Fischer, D. B. Lindenmayer, An assessment of the published results of animal relocations. Biol. Conserv. **96**, 1-11 (2000).

3. S. Godefroid, C. Piazza, G. Rossi, S. Buord, A. D. Stevens, R. Aguraiuja, C. Cowell, C. W. Weekley, G. Vogg, J. Iriondo, I. Johnson, B. Dixon, D. Gordon, S. Magnanon, B. Valentin, K. Bjureke, R. Koopman, M. Vicens, M. Virevaire, T. Vanderborght, How successful are plant species reintroductions? *Biol. Conserv.* **144**, 672–682 (2011).

4. S. Sánchez-Ramírez, Y. Rico, K. H. Berry, T. Edwards, A. E. Karl, B. T. Henen, R. W. Murphy, Landscape limits gene flow and drives population structure in Agassiz's desert tortoise (Gopherus agassizii). *Sci. Rep.* **8**, 11231 (2018).

5. D. H. Reed, R. Frankham, Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**, 230-237 (2003).

6. J. R. Chapman, S. Nakagawa, D. W. Coltman, J. Slate, B. C. Sheldon, A quantitative review of heterozygosity–fitness correlations in animal populations. *Mol Ecol.* **18**, 2746-2765 (2009).

7. M. Szulkin, N, Bierne, P. David, Heterozygosity-fitness correlations: a time for reappraisal. *Evolution* **64**, 1202-1217 (2010).

8. U.S. Fish and Wildlife Service, Endangered and threatened wildlife and plants; determination of threatened status for the Mojave population of the desert tortoise. *Federal Register* **55**, 12178-12191 (1990).

9. U.S. Fish and Wildlife Service, Desert Tortoise (Mojave Population): Recovery Plan. US Fish and Wildlife Service, Portland, Oregon (1994).

10. U.S. Fish and Wildlife Service, Revised recovery plan for the Mojave population of the desert tortoise (Gopherus agassizii). U.S. Fish and Wildlife Service, Pacific Southwest Region, Sacramento, California (2011).

11. R. W. Murphy, K. H. Berry, T. Edwards, A. M. McLuckie, A genetic assessment of the recovery units for the Mojave population of the desert tortoise, Gopherus agassizii. *Chelonian Conserv. Bi*. **6**, 229-251 (2007).

12. B. E. Hagerty, C. R. Tracy, C.R., 2010. Defining population structure for the Mojave desert tortoise. *Conserv. Genet.* **11**, 1795-1807 (2010).

13. H. B. Shaffer, E. McCartney-Melstad, P. L. Ralph, G. Bradburd, E. Lundgren, J. Vu, B. Hagerty, F. Sandmeier, C. Weitzman, C. R. Tracy, Desert Tortoises in the Genomic Age: Population Genetics and the Landscape. *bioRxiv* **195743**, doi:10.1101/195743 (2017).

14. T. Edwards, C. R. Schwalbe, D. E. Swann, C. S. Goldberg, Implications of anthropogenic landscape change on inter-population movements of the desert tortoise (Gopherus agassizii). *Conserv. Genet.* **5**, 485-499 (2004).

15. T. Edwards, K. Berry, Are captive tortoises a reservoir for conservation? An assessment of genealogical affiliation of captive Gopherus agassizii to local, wild populations. *Conserv. Genet.* **14**, 649-659 (2013).

16. R. C. Averill-Murray, B. E. Hagerty, Translocation relative to spatial genetic structure of the Mojave Desert Tortoise, Gopherus agassizii. *Chelonian Conserv. Bi*. **13**, 35-41 (2014).

17. R. Tracy, P. Medica, P. S. Corn, Translocation long-term monitoring, tortoise density evaluation, and the establishment of new LSTSs. 2006-UNR-BRRC-289-P final report to Clark County Desert Conservation Program (2006).

18. See the supplementary materials for additional detail.

19. B. A. Lawrence, T. N. Kaye, Reintroduction of Castilleja levisecta: effects of ecological similarity, source population genetics, and habitat quality. *Restor. Ecol.* **19**, 166-176 (2011).

20. M. C. Yates, D. J. Fraser, (2014). Does source population size affect performance in new environments? *Evol. Appl.* **7**, 871-882 (2014).

21. M. C. Yates, E. Bowles, D. J. Fraser, Small population size and low genomic diversity have no effect on fitness in experimental translocations of a wild fish. *Proc. R. Soc. B* **286**, 20191989 (2019).

22. G. Bowman, C. Perret, S. Hoehn, D. J. Galeuchet, M. Fischer, Habitat fragmentation and adaptation: a reciprocal replant–transplant experiment among 15 populations of Lychnis flos-cuculi. *J. Ecol.* **96**, 1056-1064 (2008).

23. C. G. Oakley, Small effective size limits performance in a novel environment. Evol. Appl. **6**, 823-831 (2013).

24. B. Hansson, L. Westerberg, On the correlation between heterozygosity and fitness in natural populations. *Mol. Ecol.* **11**, 2467-2474 (2002).

25. L. J. Allison, A. M. McLuckie, Population trends in Mojave desert tortoises (Gopherus agassizii). *Herpetol. Conserv. Biol.* **13**, 433-452 (2018).

26. https://www.drought.gov/drought/states/nevada. (accessed October 8, 2020).

27. C. C. Peterson, Different rates and causes of high mortality in two populations of the threatened desert tortoise Gopherus agassizii. *Biol. Conserv.* **70**, 101-108 (1994).

28. K. M. Longshore, J. R. Jaeger, J. M. Sappington, Desert tortoise (Gopherus agassizii) survival at two eastern Mojave desert sites: death by short-term drought? *J. Herpetol.* **37**, 169-177 (2003).

29. J. E. Lovich, C. B. Yackulic, J. Freilich, M. Agha, M. Austin, K. P. Meyer, T. R. Arundel, J. Hansen, M. S. Vamstad, S. A. Root, Climatic variation and tortoise survival: Has a desert species met its match? *Biol. Conserv.* **169**, 214-224 (2014).

30. J. Sambrook, D. W. Russell, Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (2001).

31. N. J. Bayona-Vásquez, T. C. Glenn, T. J. Kieran, T. W. Pierson, S. L. Hoffberg, P. A. Scott, K. E. Bentley, J. W. Finger Jr., L. S. Swarnali, N. Troendle, P. Díaz-Jaimes, R. Mauricio, B. C. Faircloth, Adapterama III: Quadruple-indexed, double/triple-enzyme RADseq libraries (2RAD/3RAD). *PeerJ* **7**:e7724 https://doi.org/10.7717/peerj.7724 (2019).

32. B. K. Peterson, J. N. Weber, E. H. Kay, H. S. Fisher, H. E. Hoekstra, Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one* **7**, e37135 (2012).

33. A. B. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120 (2014).

34. E. Aronesty, ea-utils: Command-line tools for processing biological sequencing data. https://github.com/ExpressionAnalysis/ea-utils (2011).

35. M. Tollis, D. F. DeNardo, J. A. Cornelius, G. A. Dolby, T. Edwards, B. T. Henen, A. E. Karl, R. W. Murphy, K. Kusumi, The Agassiz's desert tortoise genome provides a resource for the conservation of a threatened species. *PloS one* **12**, e0177708 (2017).

36. H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*, 1303.3997v1 (2013).

37. H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N Homer, G. Marth, G. Abecasis, R. Durbin, 1000 Genome Project Data Processing Subgroup, The Sequence alignment/map (SAM) format and SAMtools. *Bioinformatics* **25**, 2078-9 (2009).

38. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: analysis of next generation sequencing data. *BMC bioinformatics* **15**, 356 (2014).

39. D. A. Eaton, PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics* **30**, 1844-9 (2014).

40. R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. URL https://www.R-project.org/ (2019).

41. M. Foll, O. Gaggiotti, A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180**, 977-993 (2008).

42. M. Foll, M.C. Fischer, G. Heckel, L. Excoffier, Estimating population structure from AFLP amplification intensity. *Mol. Ecol.* **19**, 4638-4647 (2010).

43. M. C. Fischer, M. Foll, L. Excoffier, G. Heckel, Enhanced AFLP genome scans detect local adaptation in high-altitude populations of a small rodent (Microtus arvalis). *Mol. Ecol.* **20**, 1450-1462 (2011).

44. M. Plummer, N. Best, K. Cowles, K. Vines, CODA: Convergence Diagnosis and Output Analysis for MCMC, *R News* **6**, 7-11 (2006).

45. A. Raj, M. Stephens, J. K. Pritchard, fastSTRUCTURE: variational inference of population structure in large SNP data sets. *Genetics* **197**, 573-589 (2014)

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conceptualizing the main experiment based on sequencing LSTS tortoises. LJA and KJF coordinated collection of LSTS samples and identification of tortoise histories. PAS conducted all laboratory preparation, bioinformatics processing and analyses. PAS and HBS wrote the manuscript with input from RCA-M, LJA and KJF; **Competing interests:** Authors declare no competing interests; and **Data and materials availability:** All sequence data are available in the NCBI Short Read Archive (SRA accession: PRJNA638160). Data on yearly tortoise releases and resightings are available in the supplementary text: "Population density estimates in the LSTS and surrounding regions". This data is accompanying with additional information regarding pre-LSTS population estimates, regional tortoise declines, and our understanding that long-term drought is a causal force in these declines.

Supplementary Materials:

Materials and Methods

Supplementary Text

Figures S1-S8

Tables S1-S2

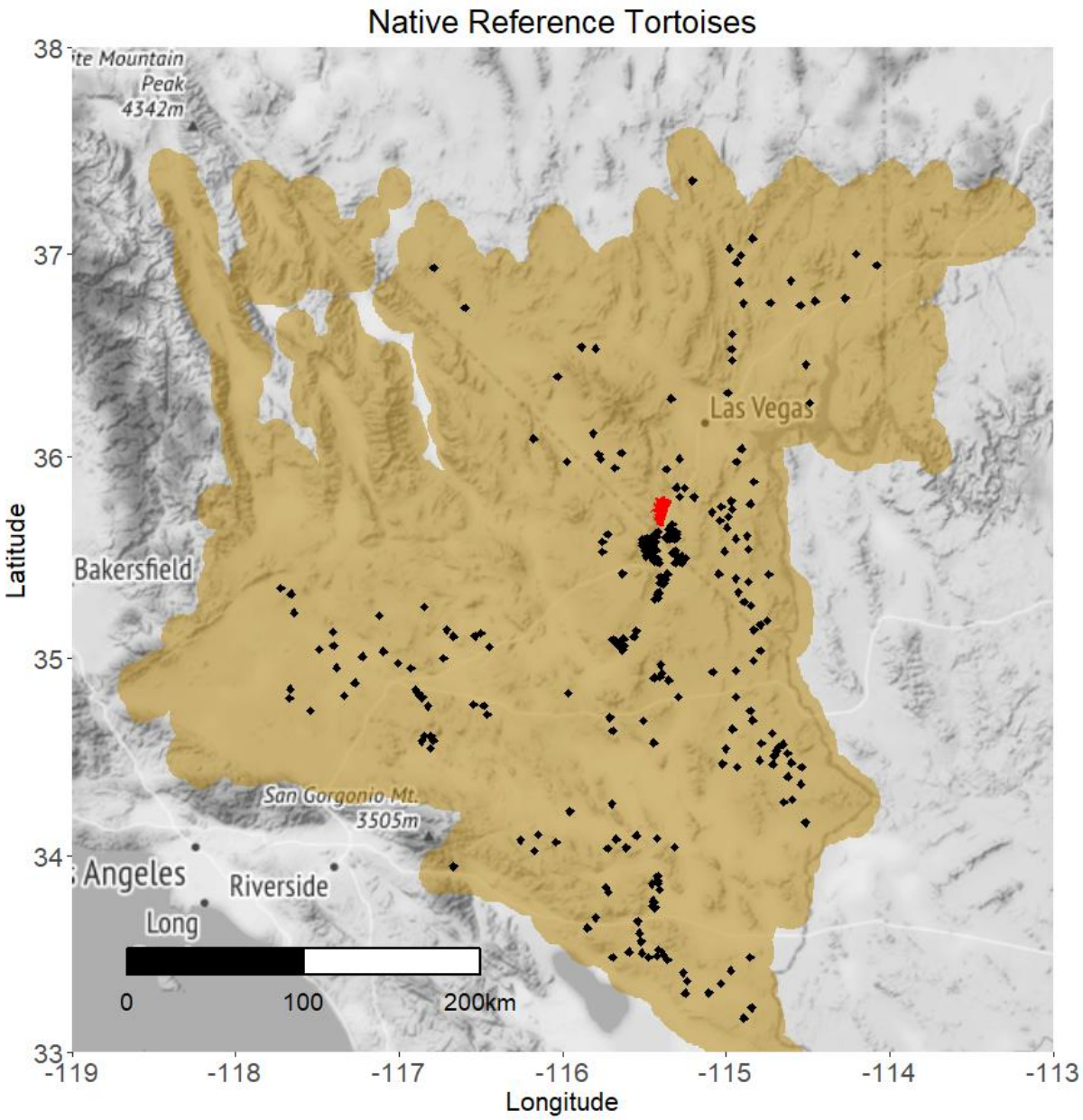

Figure 1. Map showing the approximate historic distribution of the Mojave desert tortoise (*Gopherus agassizii*) in tan, the location of 270 native low-coverage genome samples (black diamonds) and the LSTS (red polygon). We lack samples for inference only from the northwest portion of the historical distribution for the species where the tortoise is now extirpated.

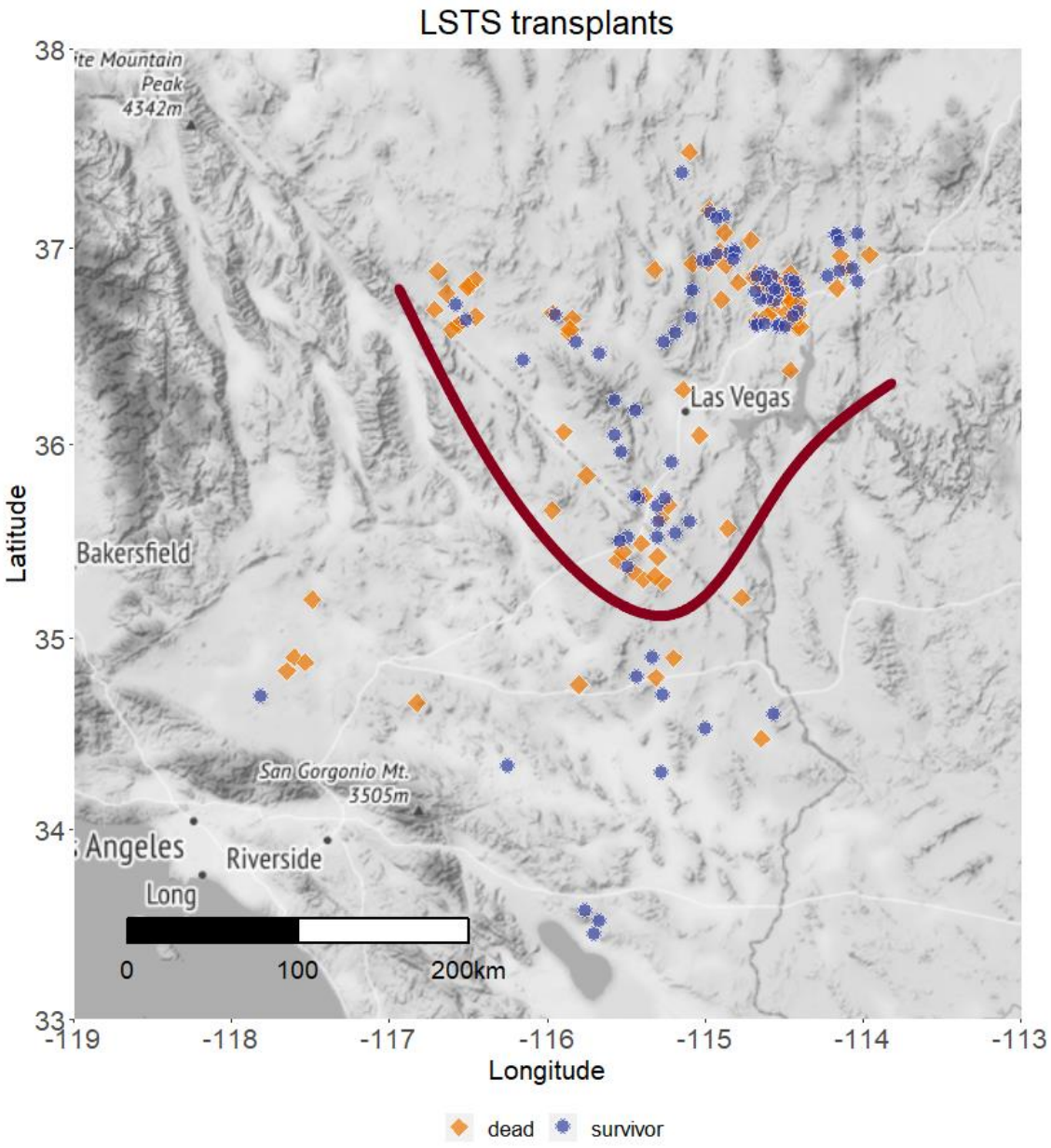

Figure 2. Map showing the inferred origins of 79 translocation survivors and 87 sex- and release year-matched translocated dead tortoises. Points are slightly jittered for visual clarity. The red line shows the boundary between northern and southern Mojave tortoise genetic units. The inferred points of origin of tortoises that died are shown as orange diamonds; those that survived as blue diamonds.

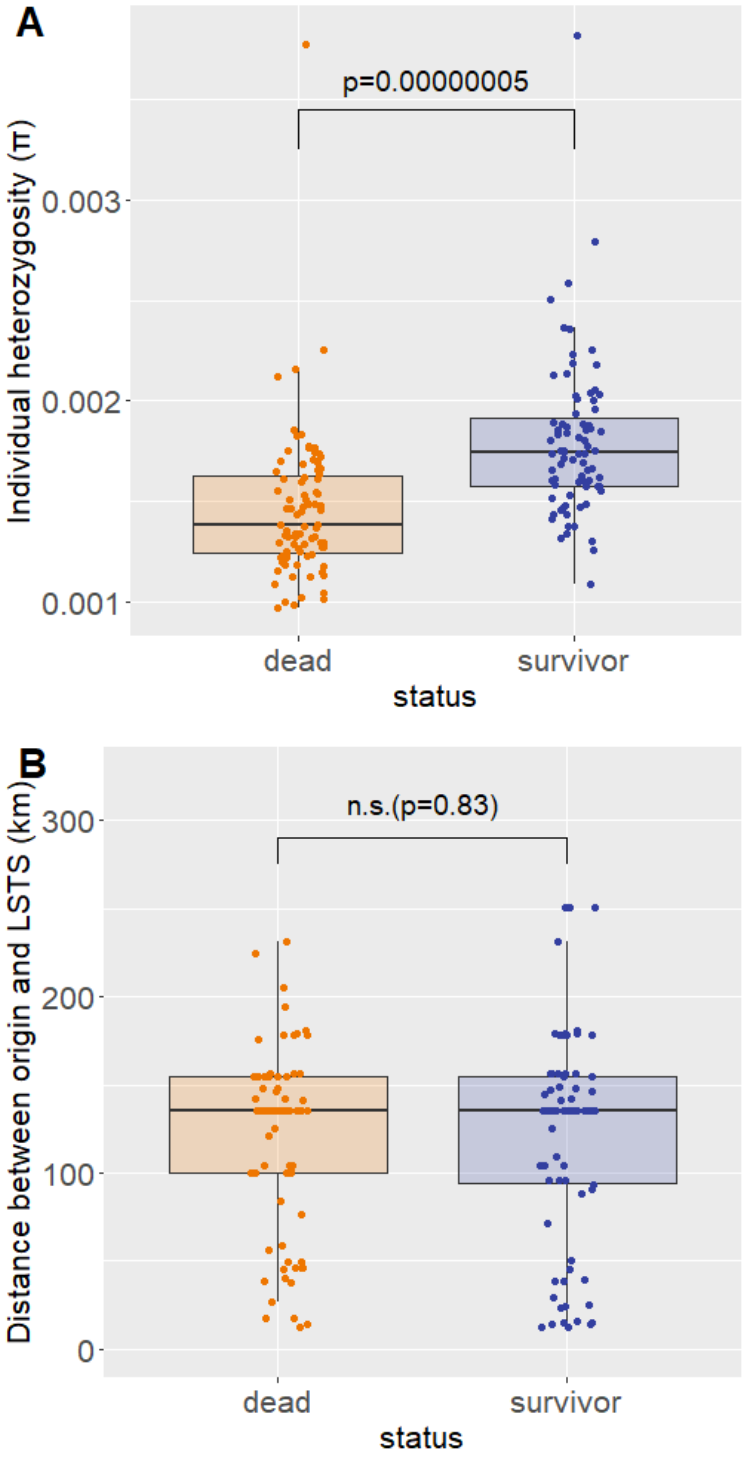

Figure 3. Box and whisker plots showing the mean and distribution of individual heterozygosity: π (A) and straight-line translocation distances (B) of LSTS mortalities and survivors. Reported p-values based on t-tests.

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This PDF file includes:

Materials and Methods

Genomic context for the LSTS tortoises

As part of our ongoing work on Mojave desert tortoise conservation genomics we recently sequenced 270 individual animals with low-coverage (~1.5x) full genome sequencing, which represents the most exhaustive geographic and genomic-scale population genetics analysis of natural genetic diversity for the species (Fig. 1; *13*). Given this resource and the availability of high-quality blood samples for almost all LSTS tortoises that were collected from tortoises prior to their release, our first goal was to generate RADseq data for LSTS individuals to infer their geographic origins. By comparing the translocated LSTS animals, including both those that lived and those that died, to the landscape-level genomic variation found in the 270 genome tortoises, we developed a cost-effective genome-scale method that allowed us to infer the site of origin of translocated individuals (Supplementary Text 1).

LSTS samples

During the 2015/16 field season, 86 adult translocated animals were confirmed to be living in the LSTS, 79 of which had blood taken and archived (either in the field or prior to release). For the remaining seven animals, no blood sample was available for genetic analysis. In addition, 71 unmarked adult tortoises were encountered within the LSTS that were believed to be part of the resident tortoise population of the LSTS. Exhaustive line distance surveys estimated that 346 (95% CI = 251.8, 475.1) adult tortoises, both native and translocated, were alive within the LSTS. Based on these population counts, we infer that translocated animals outnumber natives by about 1.2:1 (86:71), and therefore that the population of 346 tortoises currently in the LSTS consists of ~190 translocated and ~156 resident tortoises (18). We also note that our set of available blood samples, which comprises 41.6% (79/190) of the LSTS surviving translocated population, constitutes a reasonable sample of the total genetic diversity within the LSTS.

We compiled metadata for the release year and sex of the 79 known LSTS translocation survivors for which blood was available and all LSTS translocatees that were known to have died within the LSTS (verified with marked carcasses/shell remnants collected in the field) or that had not been found in recent surveys and could reasonably be assumed to be dead. We paired LSTS living and dead tortoises by release year and sex. When living LSTS individuals could not be fully paired with dead animals, we filled the paired design with animals that were presumed dead because they had not been observed in any survey since their release including recent annual surveys. We feel confident of this presumption because: 1) the overall very high mortality observed in translocated tortoises implies that most unaccounted for individuals are dead; 2) our 2015 field surveyors observed about 157 adult tortoises (55% of which were translocated), which accounts for approximately half of the total population estimated to be contained within the LSTS, suggesting that we have a high fraction of the currently living tortoises accounted for; and 3) surveys recover many carcasses and shell remains that cannot be identified because they are heavily scavenged or mostly consumed. We also note that there is no statistical difference in the heterozygosity of those tortoises that are known to be dead and those presumed to be dead ($P = 0.266$), as would be expected if they presumed dead have actually perished. As an additional check, the heterozygosity of the 56 tortoises that are known to be dead is significantly lower than those known to have survived ($P = 0.00000000016$); our primary result holds with or without the presumed dead individuals. In total we sequenced 56 dead LSTS and 31 presumed-dead LSTS transplants (87 total) and 79 living LSTS animals.

We also assembled 12 tortoise blood samples, six of which were also included in the 270 genome samples, that were field-collected, had known geographic origins, and represent the full range of natural genetic and geographic diversity of the Mojave desert tortoise. We used these 12

samples to ground-truth our strategy to estimate the geographic origin of unknown-provenance LSTS animals based on comparisons to our genome tortoise data set (Fig. 1). We refer to these 12 tortoises as our calibration samples (see Supplementary Text 1 for more details)

RADseq and Bioinformatics

We extracted DNA from ~50µl of frozen tortoise blood using a standard salt extraction protocol (*30*). For all deceased or surviving translocated tortoises, DNA was extracted from blood taken from living individuals which was then appropriately stored at -20-80˚C in 95% EtOH. Genomic DNA was standardized to 20ng/µl, and we prepared RADseq libraries for each sample using the 3RADseq protocol (*31*), which is a variant of the dual-digest RADseq protocol (*32*), with ClaI as the common-cutting enzyme, SbfI as the rare-cutting enzyme, and MspI as the dimer-cleaving enzyme. In library preparation, each individual received a unique set of combinatorial barcodes that could be used to informatically demultiplex pooled libraries. We quantified completed libraries using a Quant-iT dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA), and pooled using equimolar proportions of each library. Pooled libraries were further reduced in complexity through size selection of library fragments that were 450 - 550 bp in length with a Pippin Prep (Sage Science, Beverly, MA). Libraries were sequenced on two 150 bp paired-end HiSeq 4000 lanes. However, the second sequencing read for each fragment (R2) failed Illumina quality control specification. We resequenced all libraries resulting in four total lanes of sequence data, of which 25% (the R2 reads from the first set of sequences) were of relatively poor quality.

We performed stringent post-sequencing data processing to remove low-quality data before mapping reads or calling variants. This is a critical step to remove sequences with overall low-confidence base calls and poor-quality bases from sequence data before any downstream

analyses take place. Data-filtering is especially important when inferences rely on rare variants (which may be important in determining the geographic origin of a sample) or with low-coverage data, both of which are features of our analyses. Any reads that failed Illumina's CASAVA filter were removed from subsequent analyses/processing, reducing the total number of reads from the failed R2 sequencing attempt. Reads were then processed using Trimmomatic 0.32 (*33*) to remove poor quality sequence bases resulting from base quality degeneration at the end of some sequences and adapter contamination from short (<150bp) library inserts. Leading 5' base pairs with a phred quality score below 7 and trailing 3' base pairs with a phred quality score below 15 were removed. Then, reads were trimmed when a four base-pair sliding window (5' to 3') had an average phred base quality below 20. After trimming, all reads less than 50bp in length were discarded.

Reference-based genomics

Following sequence trimming, we merged overlapping read pairs using fastq-join from the ea-utils toolkit (*34*). Paired and unpaired reads were separately mapped to a draft of the Mojave desert tortoise genome (*35*) with bwa mem version 0.7.16a-r1181 (*36*). Sequence alignment map (SAM) files created by bwa mem were converted to binary alignment map (BAM) files and the single and paired-end read BAM files were merged with samtools version 1.5 (*37*). Resulting BAM files were sorted by chromosome coordinate, cleaned to soft-clip alignments that extended past the end of reference contigs and rescore unmapped reads, and individual tortoise read group information was added to each BAM file with picard version 2.9.0-1-gf5b9f50-SNAPSHOT (http://broadinstittute.github.io/picard). The genomics analysis tool *angsd* (*38*), which is specifically designed to work with low-coverage sequence data, was used to filter BAM files for a list of 36,138,619 high-quality polymorphic sites from our 270 genome tortoises that could be

confidently called (p<0.000001) for LSTS samples. We then used the script cPWP (*13*; https://github.com/atcg/cPWP), which calculates the mean genomic distance between two individuals while accounting for sequence read depth, to determine the pairwise genetic distances between our complete set of LSTS, calibration sample, and the 270 genome tortoises. This resulted in a matrix of pairwise genetic distance between all LSTS and genome tortoise samples.

We originally hypothesized that the optimal approach to establish provenance for LSTS individuals would be to "place" each one at the position of the genome tortoise with which it had the smallest genetic distance (Supplementary Text 1). Using our 12 calibration samples, we discovered that 1) often this strategy performed well, placing the tortoise within 5-20 km from its true origin, and 2) a few times it was off by much more (up to 216 km in one case). To determine an optimal method for reducing error in placing the calibration samples that we would use to place the LSTS individuals on the landscape, we calculated the geographical centroids of the site of collection of increasing numbers of closest genomic relatives for the 12 calibration sample individuals until the average error for landscape placement was minimized (Supplementary Text 1). Empirically, we found that our calibration tortoises fell into two classes. Individuals with high heterozygosity tended to be more accurately placed when we used the multi-individual centroid of several tortoises, whereas those with low heterozygosity tended to be more accurately placed when we used their genomic closest relative (Supplementary Text 1). We think this is the result of comparing the deeply sequenced RADseq LSTS animals to low coverage genome samples. Because our genome samples were sequenced on average to ~1.5x, we expect to infer more false negative matches for heterozygous SNPs because low sequencing depth artificially inflates homozygosity. Thus, individuals that originated in regions of high heterozygosity should

be better placed if we use the centroid of their closest relatives to average out this genetic artifact. Using our calibration sample tortoises, we determined an empirical heterozygosity cutoff ($\pi = 0.002$) for when to use a nearest-neighbor or the centroid-based inference. In both cases, we optimized both reducing the average error and standard deviation of the error in placing the calibration animals back to their known sample location (Supplementary Text 1).

De novo RADseq locus assembly and individual heterozygosity

Because our reference-mapped genomic data for LSTS tortoises included both high- and low-coverage (representing on- and off-target RAD loci, respectively) variants, we also performed de novo RADseq locus assembly so that we could focus on high-coverage, and therefore higher confidence, variants for inference about individual heterozygosity. Cleaned fastq files were processed and assembled into RADseq loci with ipyrad 0.07.13 (39) with the within- and between-cluster thresholds at 90% similarity for individual sequence assembly and intra-individual sequence depth set to ≥12X coverage. This resulted in a data set of high-confidence variants for which heterozygous positions could be called with confidence. We used these high-coverage data only to calculate individual heterozygosity (π) by dividing the number of heterozygous sites by the total number of sequenced sites for each LSTS individual.

Inferences about translocation success

To determine whether the likelihood of a successful translocation might be predicted by either the distance from the LSTS to a tortoise's inferred site of origin or the genomic heterozygosity of the tortoise, we compared living and dead translocated tortoises. We calculated the straight-line distance of living and dead animals from their sites of origin to the geographic centroid of the LSTS to quantify the relationship between translocation distance and survival. Given that the primary genetic division across the range of the Mojave desert tortoise is a relatively sharp north-

south divide (Fig. 2; *4,13*), we also determined whether significant differences existed between the number of living and dead translocated tortoises for those derived from the southern versus northern Mojave genetic groups. Finally, we calculated individual heterozygosity for living and dead LSTS individuals to determine whether levels of individual genetic diversity predicted translocation success. Multiple additional analyses were conducted to ensure that artifacts from sequencing bias, genomic outliers, additional population structure, and age class or body size did not affect these results (Supplementary Text 2).

Supplementary Text

1. Empirically determine tortoise origins

To optimize the accuracy of using genomic data inference to determine geographic origins for tortoises of unknown provenance, we RAD-sequenced 12 tortoises that were sampled within the native distribution of desert tortoises (we refer to these as "calibration tortoises"). We selected six tortoises that were part of the 270 genomic tortoises and six that were not. For each set of six tortoises, we chose samples that we thought would be "easy" and "difficult" to place on the landscape. Without prior information or data, we predicted that individuals sampled from the center or edge of either the northern or southern Mojave clades should be "easy" to place, and those from the zone of admixture between these populations would be "difficult" (Fig. S3; see *13* for natural genetic variation). Additionally, one from each set of six came from the Ivanpah Valley, where we had dense sampling and where the LSTS is also located. We expected, based on our dense genome tortoise sampling in this region, that these would be relatively easy to place back on the landscape.

These 12 tortoises were RAD-sequenced and processed with the rest of the LSTS tortoises. We then used the script cPWP (https://github.com/atcg/cPWP; *13*), which calculates the mean genomic distance between two individuals while accounting for sequence read depth variation to determine pairwise genetic distances between our full set of tortoises, including LSTS, calibration, and the 270 genome animals. Our first indication that we could successfully compare RADseq data (for LSTS and calibration tortoises) with low-coverage genome data (from the 270 genome tortoises) is that in all cases the six resequenced genome tortoises paired most closely with themselves based on their RAD data, as they should since both data sets are derived from the same individual. For these six individuals, the value of π between resequenced and RAD estimated from cPWP was not zero, because this algorithm essentially calculates average pairwise differences between each chromosome in a sample. The estimate of π within an individual is a measure of heterozygosity, and we expect more accurate identification of heterozygous positions in our RADseq data and some level of missed variation in the low coverage (~1.5x) genome data. Thus, the same individual for each data type should be calculated

as 'different' from each other at some heterozygous sites, but still yield a much lower genetic distance to itself than any other genome tortoise. This is exactly what our data show. For the six reference tortoises that were also RAD-sequenced, cPWP: mean to themselves = 0.13284, SD = 0.00590, whereas their genetic distance to their nearest relative cPWP: mean = 0.16834, SD = 0.00605. This latter genetic distance is almost identical to that of the six new calibration samples to their nearest relative; cPWP: mean = 0.169137, SD = 0.00644.

Given this success, we next considered the best way to establish provenance for LSTS samples. Our first, and simplest approach was to 'place' each LSTS sample at the geographic location of the genome tortoise with whom it shared the lowest pairwise genomic distance. When we did this with the 12 calibration samples with known origins, we discovered that 1) often this strategy performed well, placing the tortoise within 5-20 km from its true origin (Table S1; Fig. S2), and 2) a few times the error was much greater (up to 216 km in one case). We note that for the six calibration tortoises that were also genome tortoises, we excluded their sample-match and calculated this error distance to the remaining 269 genome tortoises. Overall, placing the 12 known-origin calibration samples at the location of their genetic nearest-neighbor resulted in a mean error of 61.7 km (SD= 60.2) from their true origin (Table S1; Fig. S2).

Given the relatively high error rates for a few, but not most individuals, we next explored strategies that place individuals at the centroid of their *n* nearest genetic neighbors. Determining the optimal value of *n* is difficult, so we calculated centroids, and distance of inferred origin from true origin, for every value of *n* from one (the genetic nearest neighbor) to 270 (the centroid of all genomic samples) to empirically determine the optimal value of *n* that minimized error distance and standard deviation for the 12 calibration samples. We visualized the empirical value of error rate and its standard deviation using trumpet plots and chose *n* based on the number of nearest genetic neighbors that minimized both of these values for the calibration samples (Fig. S3). These data clearly show a mean improvement in error rates across all 12 calibration samples when placed at the centroid of relatively few (n=4-8) nearest genomic neighbors. Empirically, the optimal multi-individual, centroid-based placement resulted from using the eight closest genetic relatives (Fig. S3b). Using the centroid of the closest eight relatives reduced the mean placement error for the 12 calibration samples to 41.7 km (SD=25.0).

Although we show an average improvement when using our empirically-tuned centroid based calculation for placing tortoises onto the landscape, we also showed that for some individuals centroid-based placement was worse than nearest-neighbor placement (Fig. S2, Table S1; etort143 and etort86). We discovered that the most difficult samples were often those with highest individual heterozygosity (based on RAD data) and/or that originated near lineage contact zones (our expected "difficult" cases). To determine a cutoff for when to use a single versus top 8 centroid, we ranked the 12 calibration tortoise samples from low to high individual heterozygosity, and sequentially examined every possible value as follows. First, an error rate and standard deviation was calculated using the nearest-neighbor method for all but the most heterozygous individual, which was placed with the centroid approach. Next the same error calculation was made but with the top two most heterozygous individuals using the centroid approach and the remaining 10 the genomic nearest neighbor. We continued this until all 12 used the centroid approach, and determined the single, empirically derived value of heterozygosity that minimized the overall error in placing the calibration samples. The optimal combined

approach resulted when individuals with heterozygosity (π) values as estimated from cPWP on RAD data < 0.0020 were placed with their closest genomic match (presumably their closest relative), and individuals with π values > 0.0020 were placed at the centroid of their closest eight genetic relatives. This combined approach resulted in a mean error of 35.6 km (SD= 27.7, and we used this approach to determine the geographic origin for the LSTS samples. Importantly, no matter what method was used in placing both calibration individuals *and* LSTS tortoises, individuals never switched major genetic clade assignment (from northern Mojave region to southern Mojave, or vice versa).

2. Additional validation that individual heterozygosity drives translocation success

Several factors other than true genetic heterozygosity could be potentially confounding our observed pattern that surviving tortoises had higher heterozygosity than those that died following translocation to the LSTS. Here, we report on several quality control analyses investigating the possibility that sequencing depth, genomic outliers, additional population structure, or individual characteristics were also correlated with survivorship or heterozygosity, and therefore that they, rather than heterozygosity itself, might be the causal factors driving our observed patterns.

Sequence depth and data missingness

The ability to accurately call heterozygotes depends on sufficient sequencing depth. If living and dead tortoises differ sufficiently in RADseq sequence depth, this could account for the correlation between survival and heterozygosity. In our initial settings, we imposed a relatively stringent minimum depth threshold for RADseq data of 12x coverage/locus (most analyses use a minimum of about 6x), and all individual libraries were pooled at an equal nanomolar concentration prior to sequencing. This resulted in a very high mean sequence depth of 77.83x for dead, and 332.92x for living tortoises, both of which should be more than adequate for accurately calling heterozygous sites. We presume that this difference in raw cleaned reads and coverage between samples from tortoises that eventually lived and died is likely due to lower quality gDNA for dead tortoises. One reasonable possibility is that this is attributable to the amount of time that samples have spent in storage in freezers, which on average is longer for tortoises that died (and therefore were only sampled for blood once, before they were released) than living tortoises (for which we almost always used the newest available blood sample from field collected blood samples). If there is a correlation between read depth and years spent in freezer storage, this might contribute to the lower coverage for dead tortoises, and our results. However, we found that for both dead and living translocated tortoises, there was no significant correlation between sequence depth and observed heterozygosity, and the slope of the regression lines were very close to zero (Fig. s4).

To further explore whether the difference in read depth was an important contributor to the relationship between heterozygosity and survival probability, we also conducted three in siloco down-sampling experiments to reduce the coverage depth of living tortoise samples to that of those that died. We 1) down-sampled living individuals to at most 1.3 million random sequences (the mean number of sequences for dead tortoises); 2) down-sampled living tortoises to at most 1.0 million random sequences (approximately 75% of the mean of the raw reads for dead tortoises); and, 3) down-sampled living individuals to 15% of their original number of reads in

raw data (the proportional difference in number of raw clean reads between dead and surviving tortoises). All sequence data were processed in ipyrad as for the full dataset and resulted in mean cluster depths for living tortoises of 130.75x, 110.51x, and 127.49x respectively. These depths compare reasonably well with the depth of dead tortoises (77.83x), and the variability in approaches means that several independent tests were conducted. In all cases, living translocated tortoises continued to have significantly higher heterozygosity than dead tortoises for all read depth subsampling experiments ($P = 2.9E^{-21}$; $P = 4.4E^{-12}$; $P = 1.1E^{-12}$ respectively).

To investigate the possibility that the proportion of individual data missingness was associated with inferred heterozygosity, we conducted a two-way ANOVA where missingness and survivorship were each tested as main effects, each with two levels. Individual tortoises were divided into 'high' or 'low' missingness based on whether they were above or below the median missingness for living and dead tortoises, resulting in four possible states. The results of the two-way ANOVA indicated that survivorship was a significant predictor of heterozygosity ($F(1,165) = 33.51$, $P = 0.0000000361$), while missingness was not ($F(1,165) = 1.02$, $P = 0.314$). The interaction of heterozygosity and missingness was marginally significant ($F(1,165) = 4.20$, $P = 0.042$), indicating that the difference in heterozygosity between survivors and dead tortoises was slightly different for high and low missingness. Given the insignificant main effect of missingness, we are confident that data missingness is not biasing our inference of heterozygosity on living translocated tortoises. All analyses and figures were done in R (*40*).

Reduced library complexity

Because blood samples for dead translocated tortoises were on average stored for longer in freezers than those from living translocated tortoises, and this may be driving differences in mean read depth, we wanted to ensure that dead translocated tortoise blood samples do not also have reduced library complexity that could lead to a reduction in observed heterozygosity. First, we point out that: 1) all blood samples were collected fresh, from living tortoises and stored at -20˚C to -80˚C, and thus should suffer less from reduced library complexity than is often observed when working with ancient or highly degraded DNA; and, 2) that RAD library preparation was conducted with limited cycle PCR (6 or 8 cycle) and thus the deep sequencing depth we have should be representative of many unique DNA molecules in our final library. Nevertheless, we sought to ensure that library complexity was equal in surviving and dead translocated tortoise samples.

ddRADseq methods, like the 3RADseq protocol used here, reduced the genome using restriction enzymes, and therefore by definition putative homologous loci start and stop at the same position on the chromosome. This means that normal tools (like Picard's MarkDuplicates algorithm) cannot be used to identify, and discard PCR duplicates during variant calling. However, we postulate that if, on average, one group of tortoises (dead) was suffering from reduced library complexity at heterozygous sites more than another group (living), then the frequency of heterozygous bases should differ much more from the expected value of 1:1 (expected if both chromosomes were sequenced evenly) in the dead tortoise samples compared to the living. To examine this possibility, all biallelic heterozygous sites were extracted from iPyrad's VCF output and we calculated the ratio of the most abundantly sequenced allele to the lesser sequenced allele – we refer to this as the **het allele ratio**. Under the expectation that if each diploid chromosome was sequenced evenly at a heterozygous site, the expected value should be 1. We used the more common allele as the numerator so that the ration was always greater than or equal to one. If on average, dead translocated tortoises suffered from limited library complexity driven by a greater

proportion of sequenced PCR duplicates, we'd expect the distribution of their het allele ratio to have a thicker tail than the surviving tortoises because they are actually based on a lower sample of unique chromosomes being sequenced. Although the total read counts are lower in the DNA sequences from tortoises that eventually died (see the coverage difference described earlier), we see no indication that the two distributions are different (Fig. s5)—they are virtually identical. We conclude that dead translocation tortoises do not, on average suffer from reduced library complexity at heterozygous RAD loci.

We also directly addressed the possibility that the age of the DNA sample could be driving inferred heterozygosity. Here, we asked: do older DNA samples result in fewer heterozygous SNP calls? To do this, we simply plotted the age of DNA sample (that is, when the blood that we sequenced was drawn and stored in a freezer) against that individual's heterozygosity. Plots were made separately for translocated tortoises that lived and died and show no evidence that blood sample age is driving inferred individual heterozygosity (Fig. s6). In each case there is one clear outlier with high heterozygosity; when these two individuals were deleted, the results were unchanged.

Genomic Outliers

We investigated the possibility that specific genomic variants differentiated living and dead translocate tortoises, rather than a more general effect of overall multilocus heterozygsity, with genomic outlier analyses in BayeScan v2.1 (*41-43*). Individuals were classified into two groups (living or dead), BayeScan was run for 100,000 MCMC generations following 20 pilot runs of 500 MCMC generations, a burn-in of 50,000 generations, and a prior odds parameter that neutrality is more likely than selection of 500, which is suitable for 252,585 SNPs used in the analysis. Convergence of MCMC chains was assessed with the R package coda (*44*). We observed visual convergence of the MCMC chains (Fig. s7), effect sizes of each population that were much larger than actual size (effect size dead = 3379.83; living = 848.545), Geweke's convergence diagnostic was not significant (dead $z = 0.566$; living $z = -0.261$), and that both populations passed Heidelberg and Welch's convergence diagnostic (dead $P = 0.083$; living $P =$ 0 .35). The BayeScan analyses identified no significant outlier SNPs between dead and living translocated tortoises at a false discovery rate of 0.05. Because BayeScan can detect both directional selection and diversifying (or balancing) selection, which would be represented by loci with higher than average heterozygosity, our results indicate that overall heterozygosity, and not that of a small number of specific genomic variants is associated with translocation survival.

Additional Population Structure

To investigate if fine-scale population structure beyond the primary north/south differentiation could be driving translocation survivorship, we ran fastSTRUCTURE (*45*) to identify any additional groups in our LSTS tortoises. SNPs were thinned to one variant per RAD locus and restricted to those present in at least 90% of individuals. The number of populations found to maximize the model marginal likelihood was one, and the number of populations found to best explain population structure was four. For K=4 populations (Fig. s8), the largest group (subpopulation 1) was tortoises from the northern group (green bars in Fig. s8), and was equally distributed among living and dead tortoises (61/79 living and 76/86 dead individuals; $\chi 2 = 0.34$, df = 1, $P = .56$), supporting our inference that the bulk of translocated tortoise, regardless of status, originated in the northern Mojave metapopulation. Smaller numbers of individuals were found in the three southern-group subpopulations, again with no differences in the proportions of

living and dead animals (subpopulation 2 = 11 living, 8 dead; subpopulation 3 = 4 living, 1 dead; subpopulation 4 = 3 living, 1 dead). Although the additional structure from those individuals from the southern Mojave metapopulation indicates greater fine-scale genetic structure than the northern metapopulation (*11-13*), there does not seem to be any trend toward population genetic group membership and survivorship. Overall, this analysis supports our earlier discovery that population of origin is not a driver of translocation survival in Mojave desert tortoises.

Individual Characteristics

We analyzed two measures of individual condition, size and sexual maturity, which were taken at or near the time of tortoise release to determine any possible correlation with translocation survival. We found that most translocated tortoises were adults (defined as midline carapace length > 180mm; 77 of 79 living tortoises; 85 of 87 dead tortoises) and there was no significant correlation in adult to juvenile ratio with translocation survival ($\chi 2$ (df = 1, N = 166) = 0.75, P = 0.36). Additionally, there was no difference in mean carapace length between tortoises that lived or died after translocation (mean carapace length of living tortoises = 256.01mm, mean carapace length of dead tortoises = 263.30mm; t = -1.129; P = 0.251). These results further support our conclusion that heterozygosity, not age class or size, is the driver of translocation success in Mojave desert tortoises.

3. Population density estimates in the LSTS and surrounding regions:

Although absolute mortality schedules are not central to this study, it is important to place the high mortality observed in the LSTS in the context of tortoise population collapse in the region more generally, and **to provide the evidence supporting** our assertions that 1) 9105 tortoises were released into the LSTS between 1997 – 2014, 2) an additional 1449 tortoises existed on the LSTS site before releases began, 3) high levels of regional and species-wide mortality of desert tortoises were ongoing during this period, 4) both native and translocated animals perished within the LSTS, and 5) our sample of 79 living translocated tortoises represents roughly 38.4% of all remaining transplanted individuals, which is a reasonable sample for population inference. The density of desert tortoises has declined precipitously in both the LSTS and surrounding area. In 1996, before any releases occurred, surveys of 60 4-hectare plots estimated that there were 1449 tortoises in the area that would become the LSTS (*17*; see their Table 2, p. 8). By 2005, declines at a regional control site (Piute Valley) for the LSTS were reported at roughly 80% (*17*; p. 10-11). Further declines were also reported throughout the native distribution of the Mojave desert tortoise, with species-wide estimated declines of adult densities of 36.9% between 2004 and 2014 (*25*). If both apply to the roughly 1449 tortoises that were fenced within the LSTS, approximately 182 native tortoises remained alive in 2014.

Field work conducted by the USFWS in 2015 confirmed these estimates. Quantitative field work based on 1704 km of visually surveyed transects over 22 days resulted in 58 observations of adult living tortoises. After correcting for the fraction of tortoises missed by transect observers, these visual encounter transects resulted in an estimated 3.33 tortoises/sq.-km, or 346 adult tortoises. Of those 58 living tortoises, 28/47 were translocated individuals, 19/47 were native LSTS tortoises, and 11 could not be safely extracted from their burrows. Based on the fraction of scorable tortoises that were translocatees (59.6%), we estimate that 206 of the 346 living tortoises on the LSTS in 2015 were translocatees, and 140 were the remaining native tortoises.

Fig. S1.

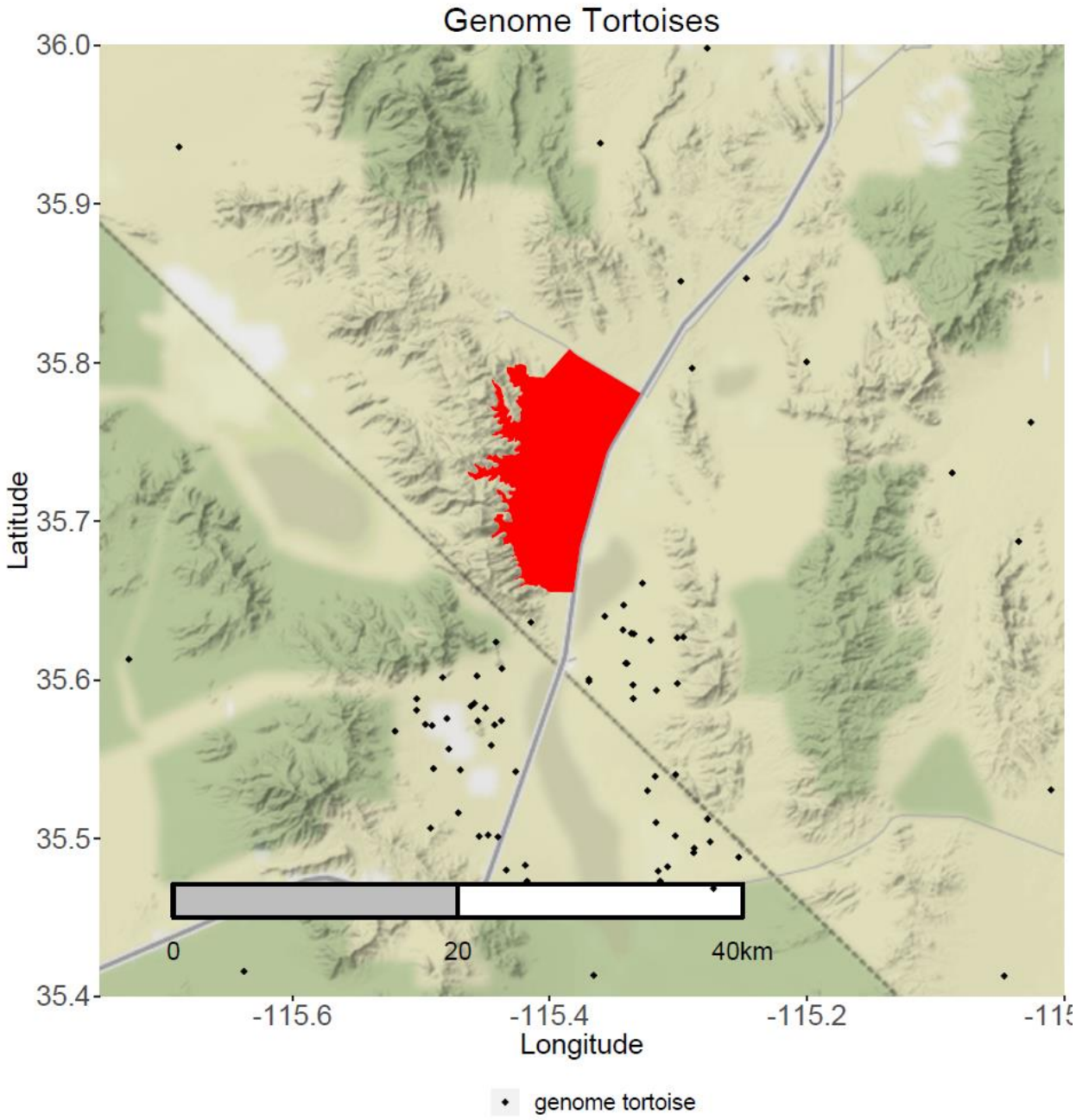
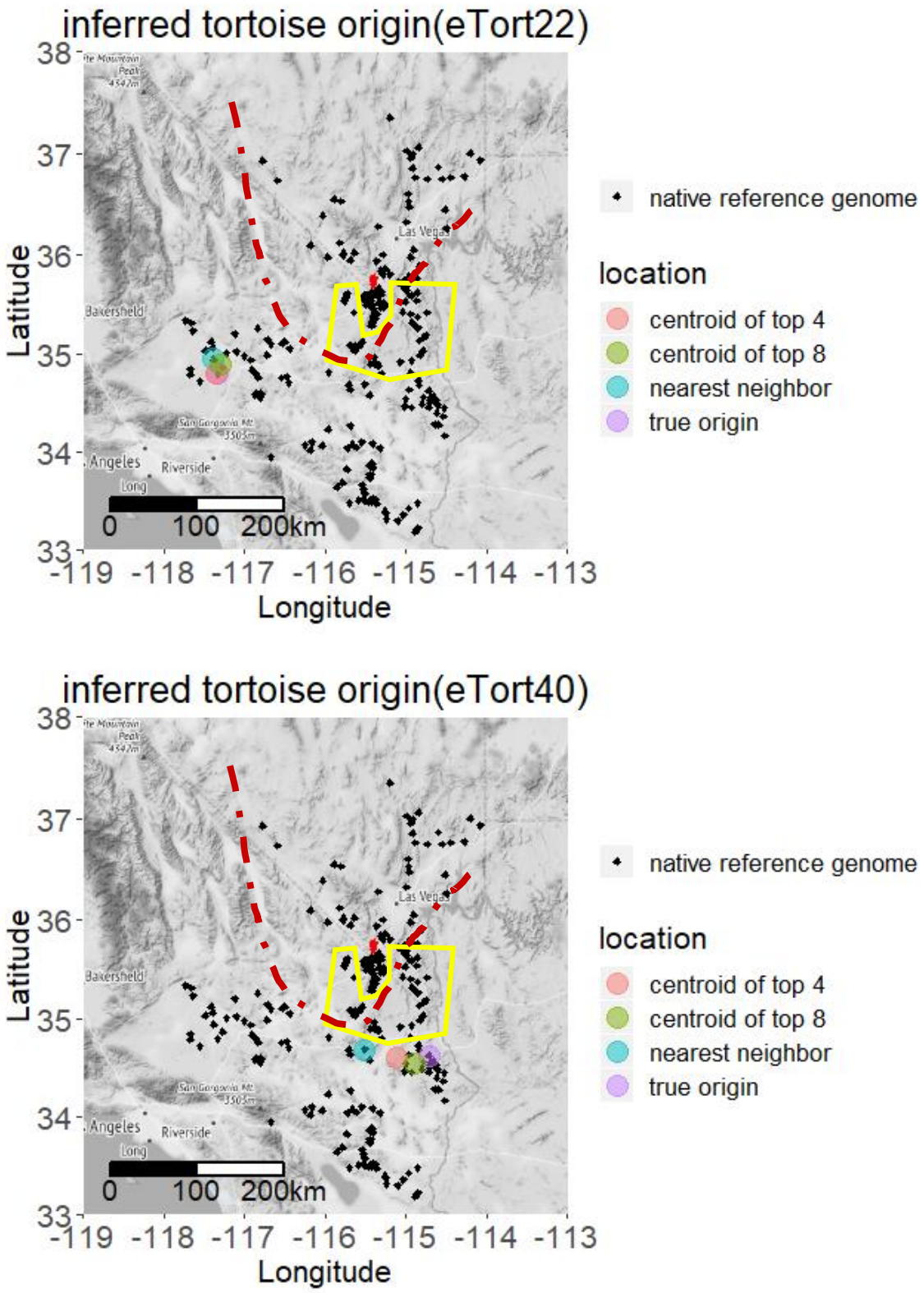


Figure S1. **Enlarged map showing the location and extent of the LSTS in red with nearby low coverage reference genomes in black.** For the complete extent of low-coverage genome samples see Fig. 1a.

Fig. S2.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Angeles
Riverside
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native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin
inferred tortoise origin(eTort40)
Latitude
Longitude
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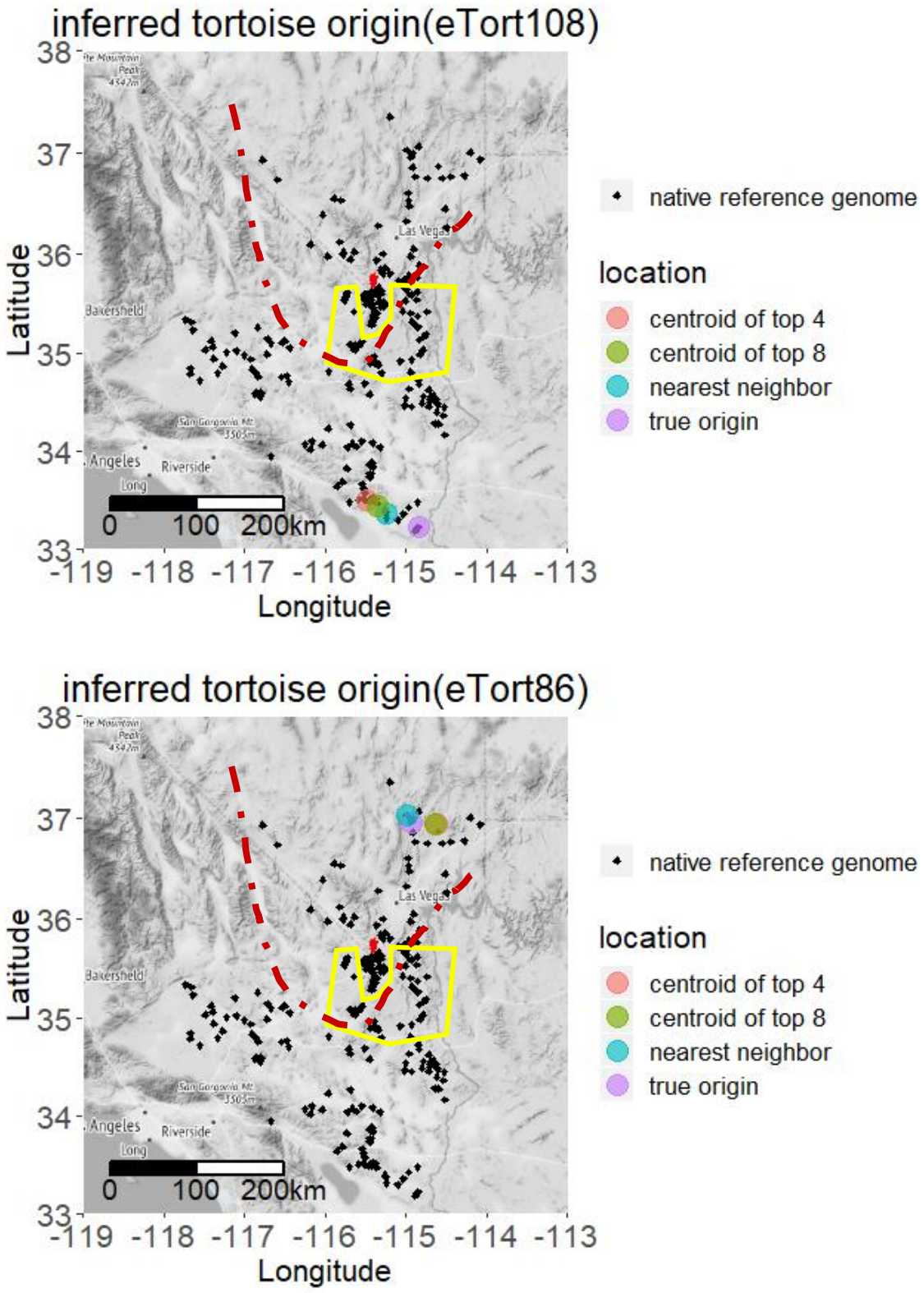
true origin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Riverside
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nearest neighbor
true origin
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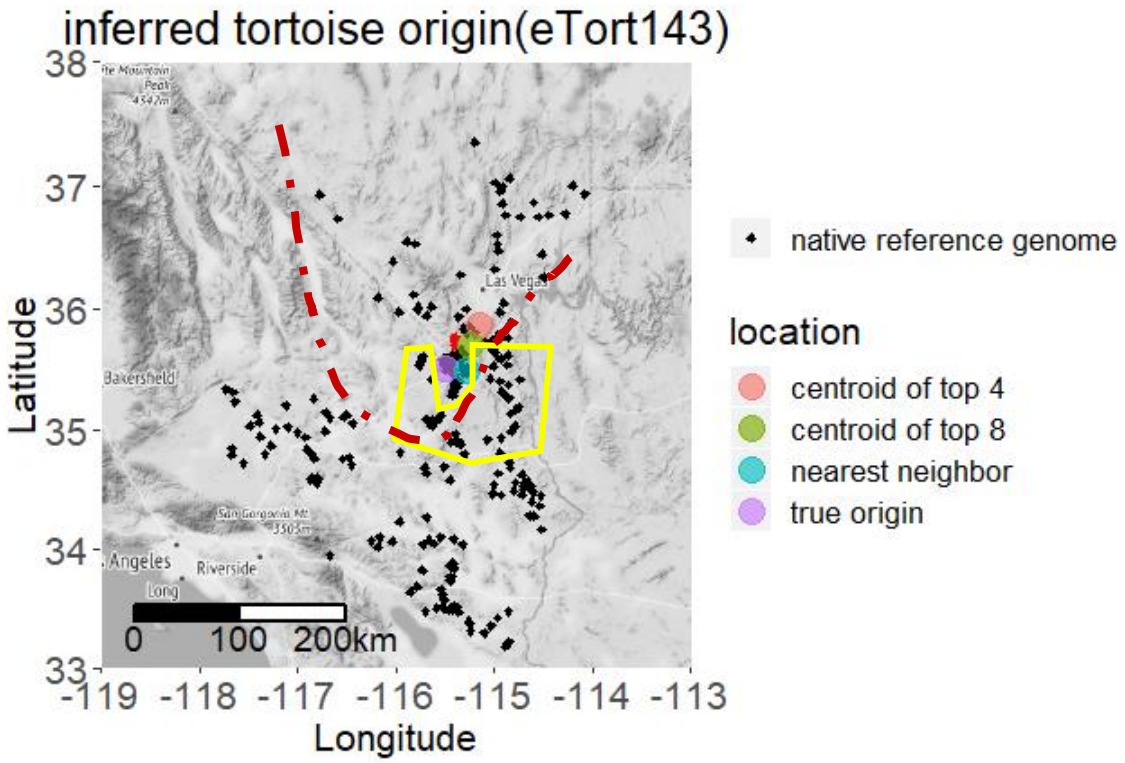
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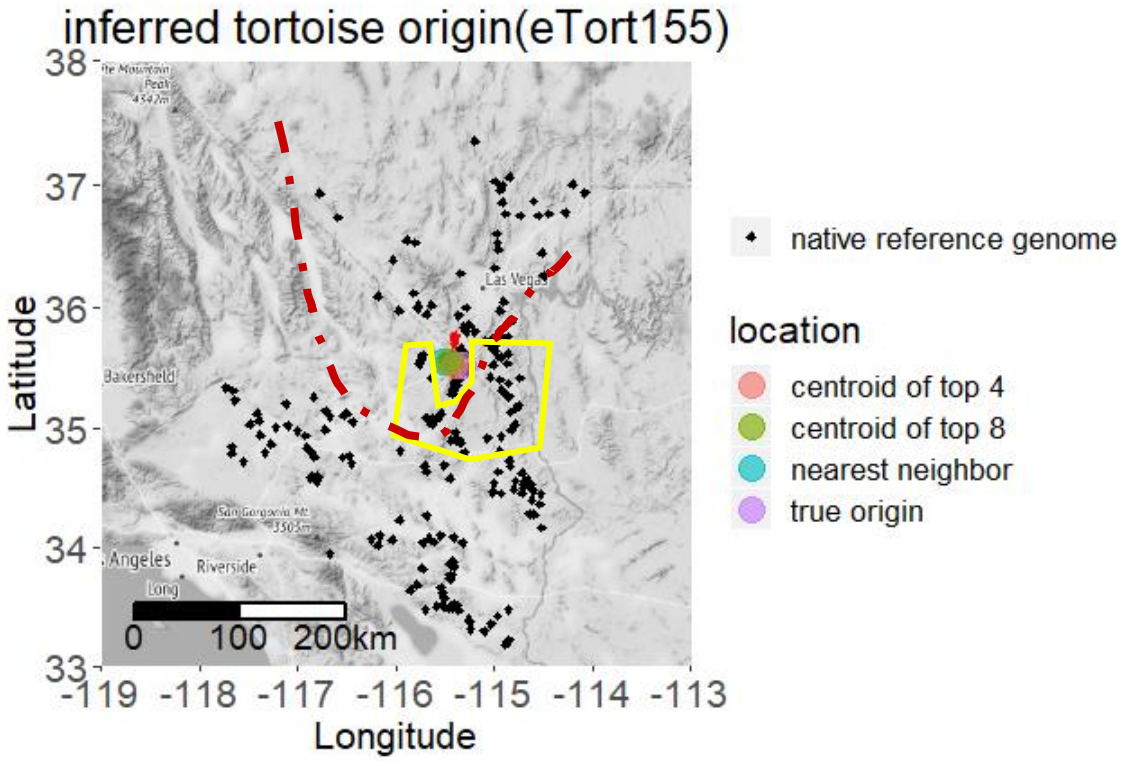
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin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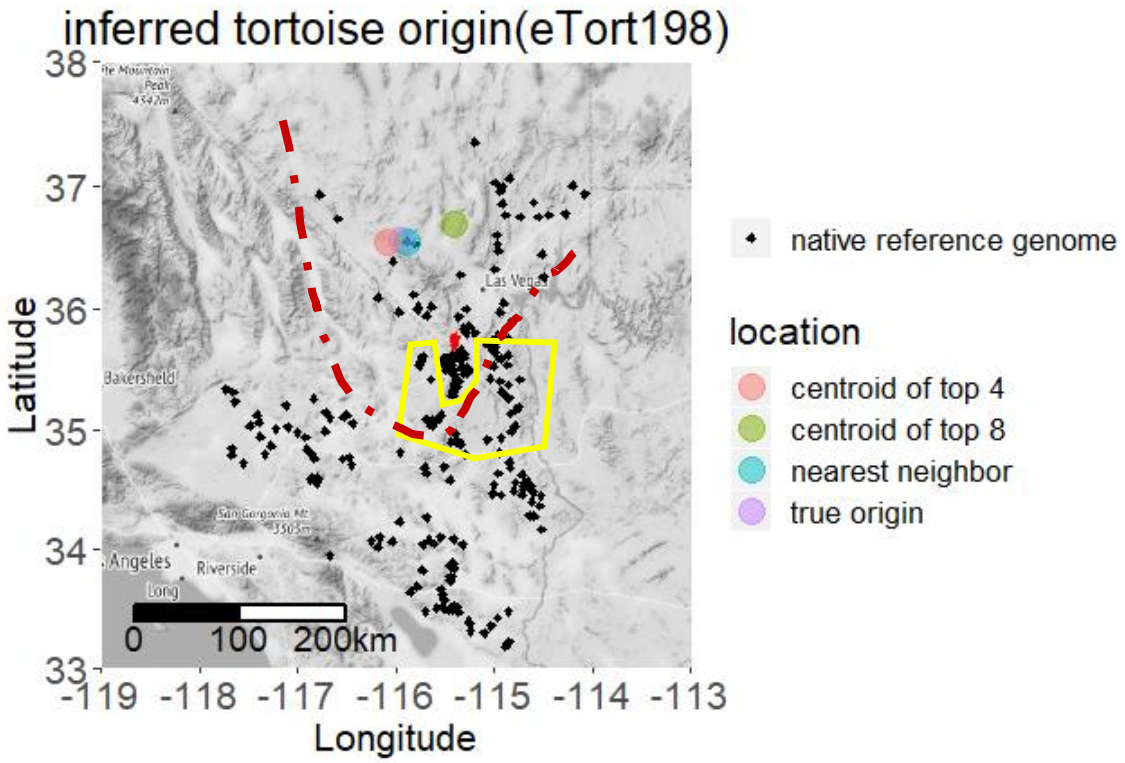
nearest neighbor
true origin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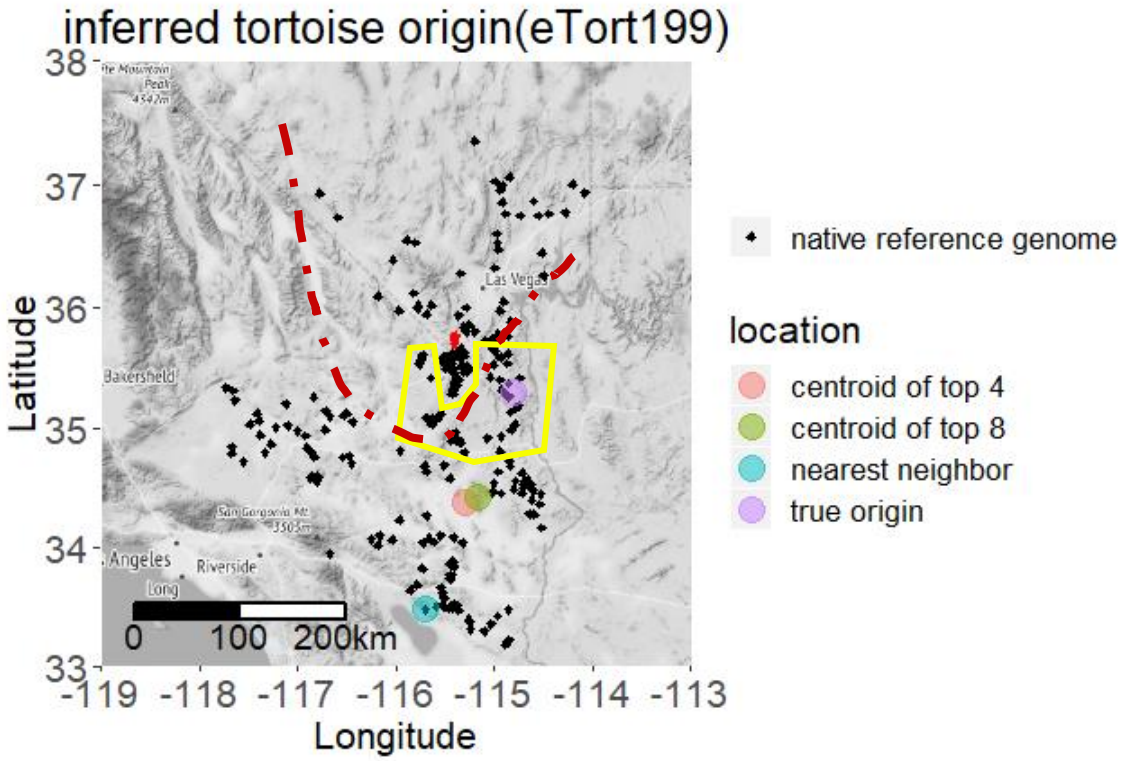
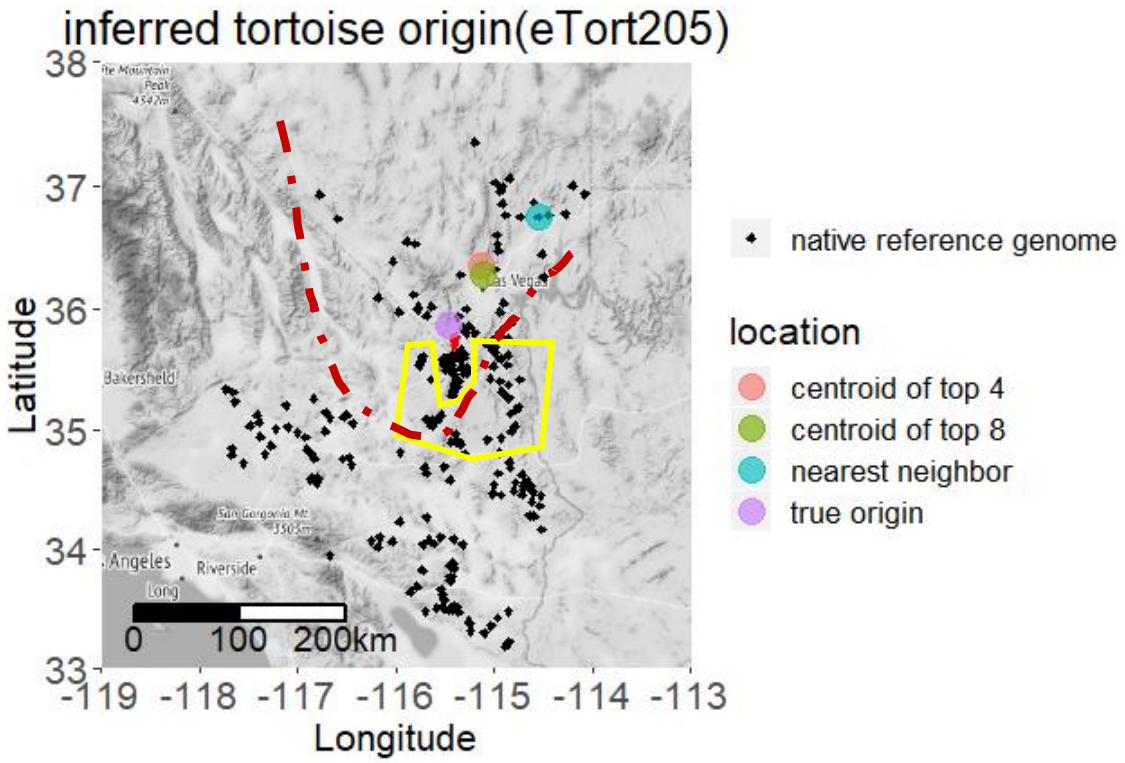
Angeles
Riverside
Long
0 100 200km
native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin


inferred tortoise origin(eTort199)
Latitude
Longitude
38
37
36
35
34
33
-119 -118 -117 -116 -115 -114 -113
Las Vegas
Bakersfield
Angeles
Riverside
Long
0 100 200km
native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin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Longitude
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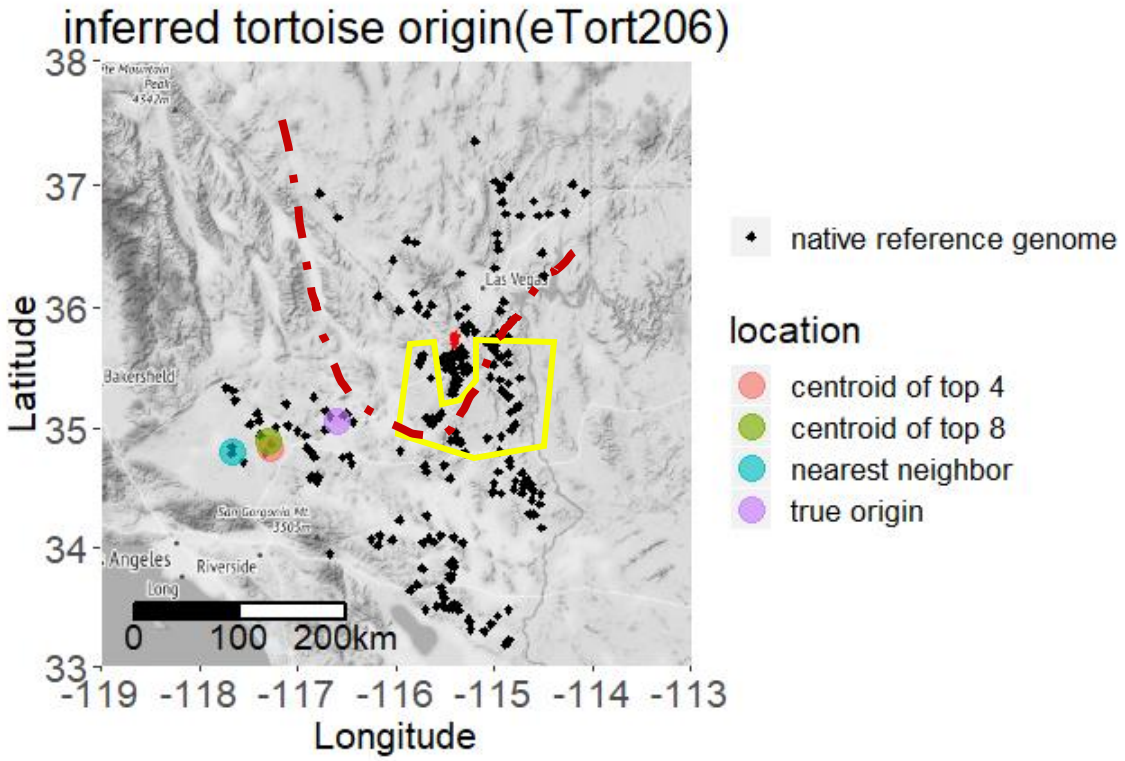
37
36
35
34
33
-119 -118 -117 -116 -115 -114 -113
Las Vegas
Bakersfield
Angeles
Riverside
Long
0 100 200km
native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin


inferred tortoise origin(eTort206)
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Longitude
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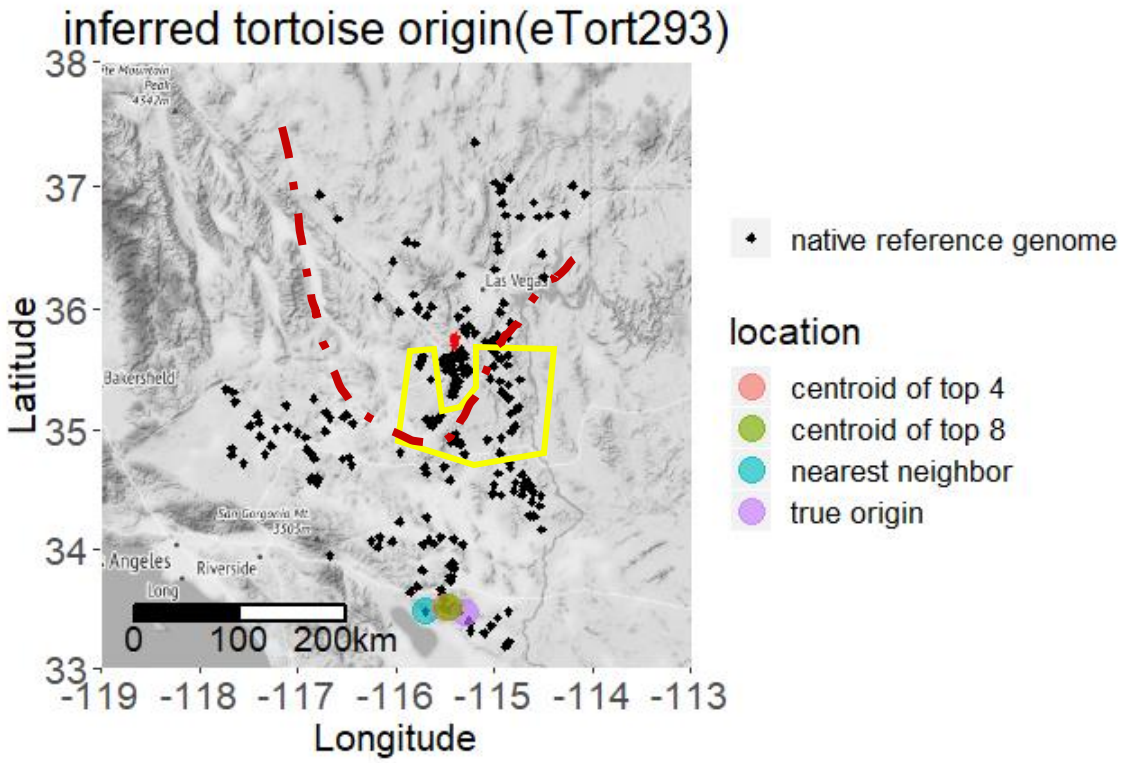
37
36
35
34
33
-119 -118 -117 -116 -115 -114 -113
Las Vegas
Bakersfield
Angeles
Riverside
Long
0 100 200km
native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin


inferred tortoise origin(eTort293)
Latitude
Longitude
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37
36
35
34
33
-119 -118 -117 -116 -115 -114 -113
Las Vegas
Bakersfield
Angeles
Riverside
Long
0 100 200km
native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin

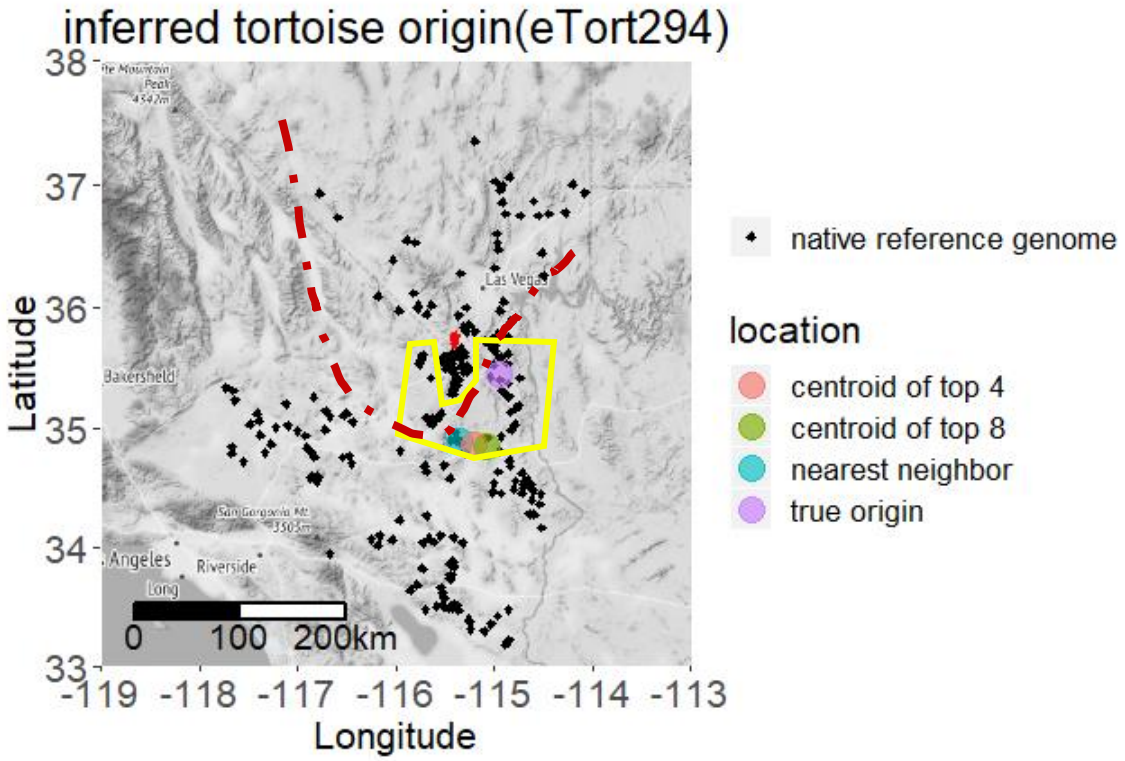
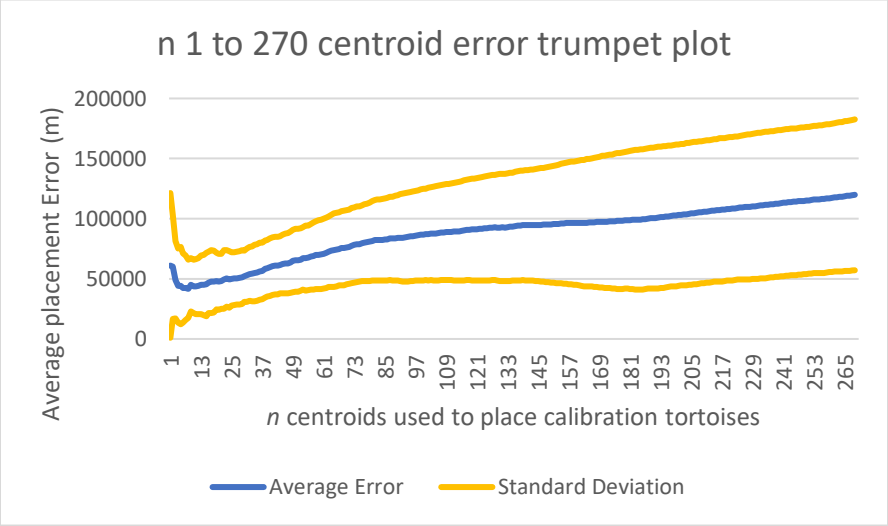

inferred tortoise origin(eTort294)
Latitude
Longitude
38
37
36
35
34
33
-119 -118 -117 -116 -115 -114 -113
Las Vegas
Bakersfield
Angeles
Riverside
Long
0 100 200km
native reference genome
location
centroid of top 4
centroid of top 8
nearest neighbor
true origin

Figure S2. Maps showing sampling locations, locations of the inferred nearest neighbor, and locations of the centroids of the top four or eight nearest neighbors for the 12 calibration tortoises. Locations of all 270 genome tortoises are shown in black and the LSTS in red. The division between northern and southern genetic populations of Mojave desert tortoises is shown in the dashed line and approximate region of admixture between these units from which we predicted tortoises would be "difficult" to place is shown in the yellow polygon. When locations for all four inference methods are not visible, multiple methods inferred locations at virtually the same place, so locations overlap. Inference of native genetic variation comes from (*13*).

Fig S3.

a



b

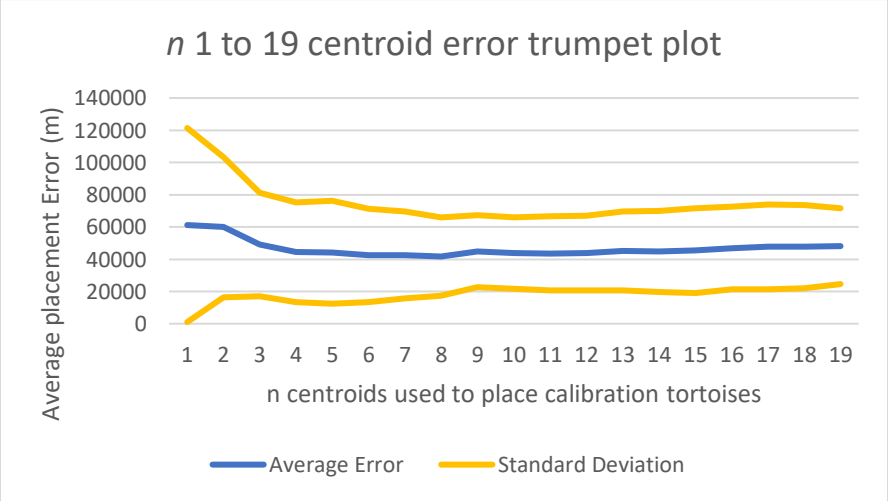


Figure S3. Trumpet plots showing the improvement and then decay in inferred placement error rates and standard deviations for 12 calibration tortoises for all data (a) or zooming in on the first *n* 1 to 19 nearest neighbors used in centroid calculation.

Fig S4.

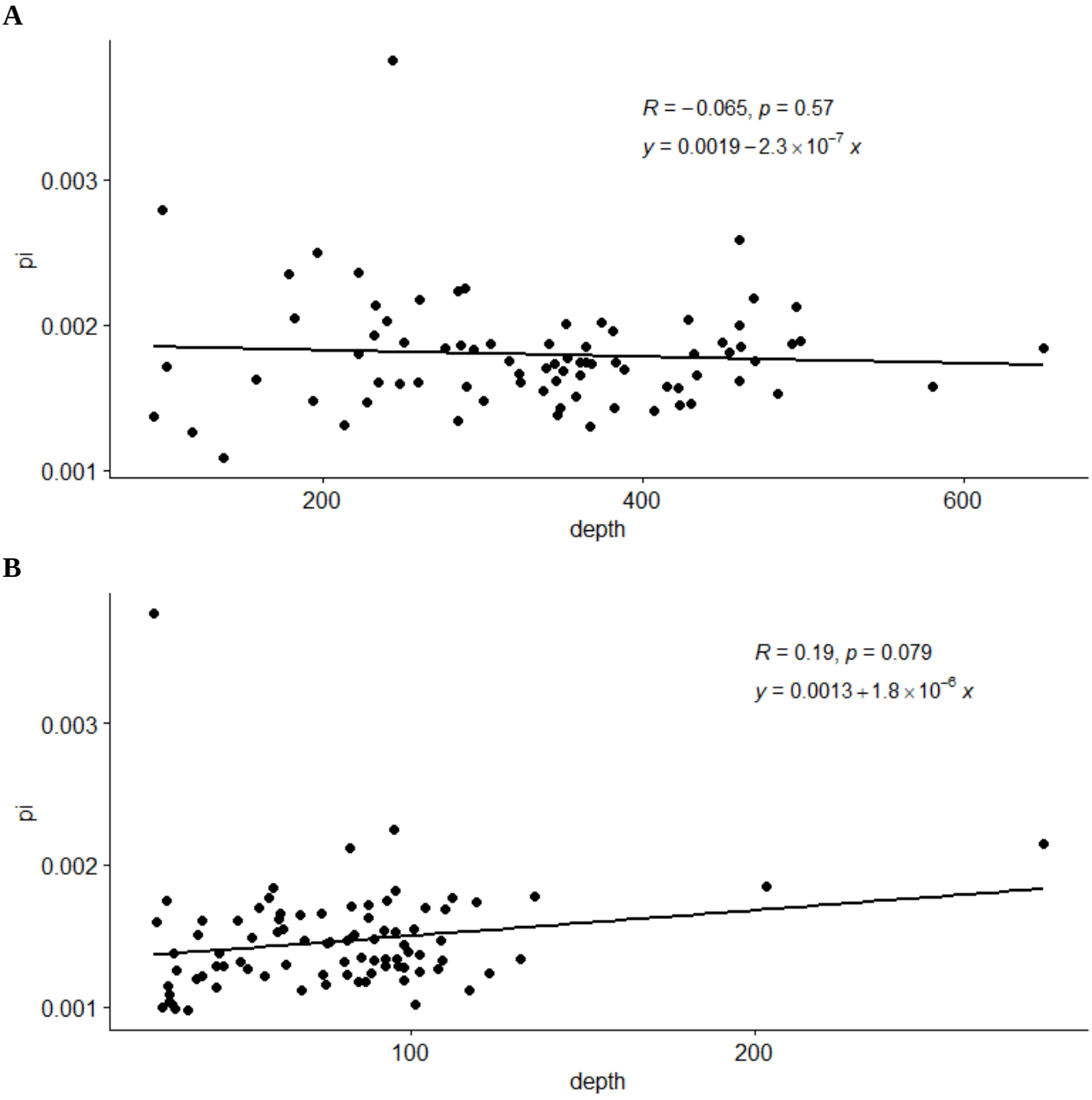


Figure S4. Scatter plots showing the distribution of individual heterozygosity (pi) by mean read depth per individual and a linear regression for this distribution for a) living translocated tortoises b) dead translocated tortoises.

Fig S5

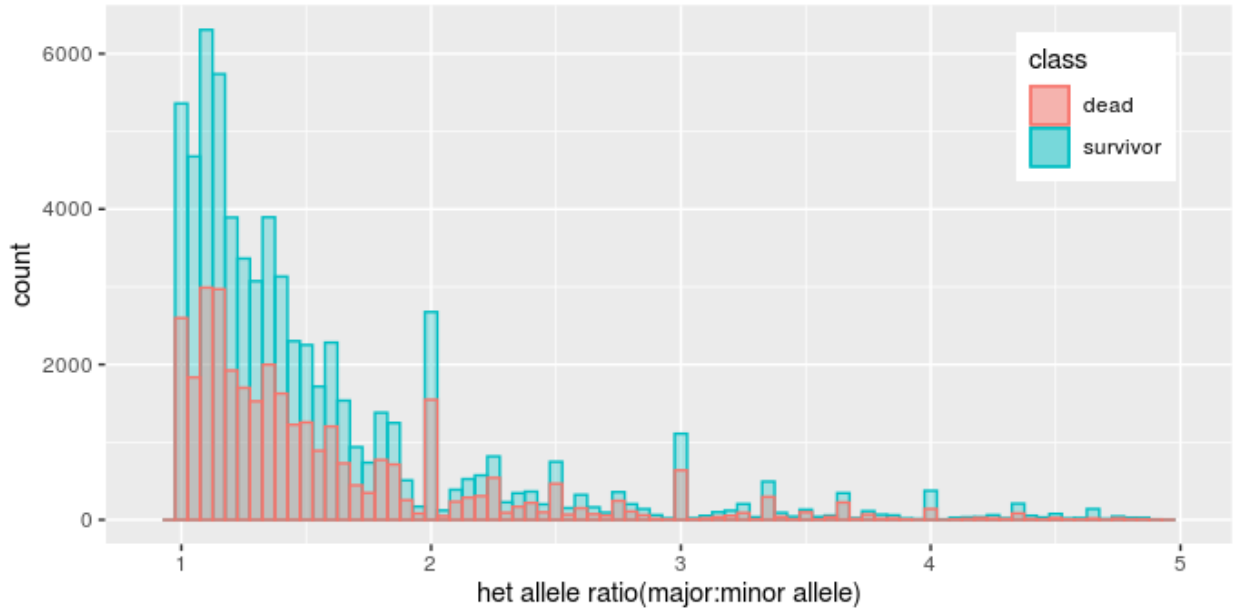


Figure S5. Histogram showing the ratio of major to minor allele read counts at heterozygous sites for dead and living translocated tortoises. We show these as overlayed, not stacked, distributions, with the dead tortoise distribution overlaying survivors. These distributions do not indicate that translocated tortoises that died have lower library complexity that might be causing missed heterozygous SNP calls. Rather, average library complexity is essentially identical for translocated tortoises that eventually lived and died post translocation.

Fig S6.

Figure S6. Graphs showing individual heterozygosity plotted against blood sample collection date (the source of the DNA that was eventually sequenced) for dead (top) and surviving (bottom) translocated tortoises. Both graphs show an essentially flat relationship between heterozygosity and blood sample date indicating that the older blood samples for translocated tortoises that died is not associated with variation in heterozygosity.

Fig S7.

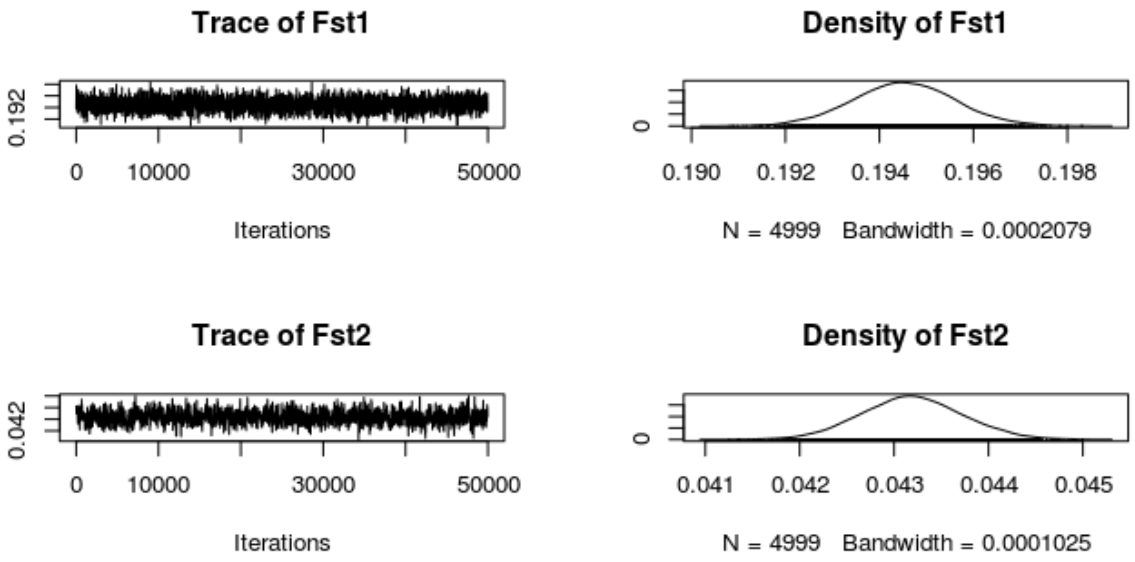


Figure S7. Trace plots and density distributions of population-level F_{st} estimates showing convergence of BayeScan analyses. $F_{st}1$ are dead translocated tortoises and $F_{st}2$ is for living translocated tortoises.

Fig S8.

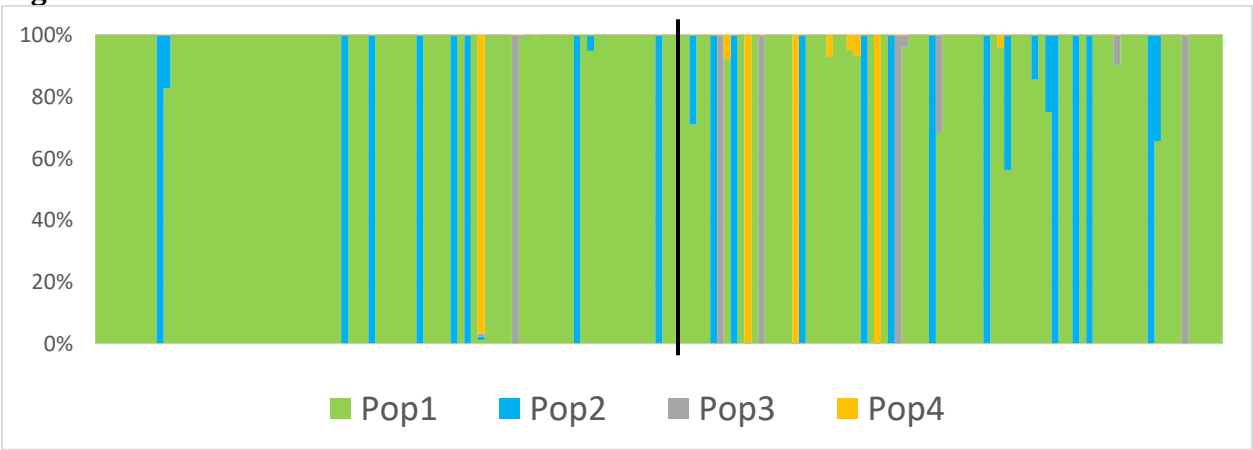


Figure S8. A plot of fastSTRUCTURE population groupings for translocated tortoises. Each bar represents the proportional ancestry of an individual to any of K=4 populations. The green individuals (Pop1) are northern group tortoises, and the blue, gray and yellow are three additional grouping from the southern group tortoises. The black bar separates dead translocated tortoises (left) from surviving translocated tortoises (right).

Table S1.

	inferred error rate (km)			heterozygosity
Sample	single neighbor	top 4 centroid	top 8 centroid	estimated π
etort22	16.34855483	1.882477905	11.03642514	0.001939704
etort40	72.9196321	35.66790954	17.16736669	0.002062541
etort86	8.366912035	29.07230931	30.71580985	0.001493187
etort108	39.71637632	67.02289745	55.10054421	0.002265407
etort143	19.37969685	50.29400877	34.23413606	0.001812372
etort155	17.26396438	7.369777579	20.44067161	0.001831052
etort198	4.722561275	12.83411141	42.98018328	0.001776651
etort199	216.980238	110.5528677	95.82395171	0.002268596
etort205	128.345277	62.90082083	47.58984685	0.002231965
etort206	101.8973573	66.67290415	55.84818679	0.002123177
etort293	36.09482014	18.70633258	16.97414459	0.002096062
etort294	72.17065376	69.04174938	72.21275446	0.002194614

Table S1. Individual error rates in placing 12 calibration tortoises back to their known sampling location on the landscape. Some individuals always have a small error rate, others consistently have greater error. For those with the largest single-neighbor errors (etort199, etort205, etort206) we reduce error rates by placing them at the centroid of eight nearest neighbors. Heterozygosity, based on RADseq data, for each calibration sample is shown which demonstrates the general reduction in error in placing individuals with high heterozygosity at the centroid of their eight nearest neighbors.

Table S2.

	Adult translocatees			Juvenile translocatees		
Release Year	Released	Resighted (Live)	Recovered (Dead)	Released	Resighted (Live)	Recovered (Dead)
1997	236	6	16	405	6	6
1998	420	29	60	461	9	8
1999	198	19	31	375	10	9
2000	98	6	12	66		1
2001	627	21	99	799	18	18
2002	138	6	21	33		1
2003	377	26	81	605	11	11
2004	205	12	48	307	6	2
2005	459	23	127	399	5	13
2006	318	8	77	137	4	5
2007	239	5	63	256	3	4
2008	312	21	58	226	3	5
2009						
2010	78	8	4			
2011	443	33	44	73	1	
2012	339	26	11	332		1
2013						
2014	87	23	3	56		
total	4574	272	755	4531	76	84

Table S2. The number of adult (at least 180 mm maximum carapace length, or MCL) and juvenile tortoises released each year, and the number from that yearly cohort ever resighted or recovered dead. Most resightings reported before 2010 and many reported starting in 2010 were incidental to other activities rather than part of formal surveys. In total, 9105 tortoises were released.